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(54) Title: OLIGONUCLEOTIDE ANALOGUES																											
<p style="text-align: center;"> V X Y Z </p> <p> ZT: B = thymin-1-yl ZU: B = uracil-1-yl ZG: B = guanin-9-yl ZC: B = cytosin-1-yl ZA: B = adenin-9-yl ZMeC: B = 5-methylcytosin-1-yl </p>																											
(57) Abstract The present invention relates to novel bicyclic and tricyclic nucleoside and nucleotide analogues as well as to oligonucleotides comprising such elements. The nucleotide analogues, LNAs (Locked Nucleoside Analogs), are able to provide valuable improvements to oligonucleotides with respect to affinity and specificity towards complementary RNA and DNA oligomers. The novel type of LNA modified oligonucleotides, as well as the LNAs as such, are useful in a wide range of diagnostic applications as well as therapeutic applications. Among these can be mentioned antisense applications, PCR applications, strand displacement oligomers, as substrates for nucleic acid polymerases, as nucleotide based drugs, etc. The present invention also relates to such applications.																											

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OLIGONUCLEOTIDE ANALOGUES**FIELD OF THE INVENTION**

- 5 The present invention relates to the field of bi- and tricyclic nucleoside analogues and to the synthesis of such nucleoside analogues which are useful in the formation of synthetic oligonucleotides capable of forming nucleobase specific duplexes and triplexes with single stranded and double stranded nucleic acids. These complexes exhibit higher thermostability than the corresponding complexes formed with normal
- 10 nucleic acids. The invention also relates to the field of bi- and tricyclic nucleoside analogues and the synthesis of such nucleosides which may be used as therapeutic drugs and which may be incorporated in oligonucleotides by template dependent nucleic acid polymerases.

15 BACKGROUND OF THE INVENTION

Synthetic oligonucleotides are widely used compounds in disparate fields such as molecular biology and DNA-based diagnostics and therapeutics.

20 Therapeutics

- In therapeutics, e.g., oligonucleotides have been used successfully to block translation in vivo of specific mRNAs thereby preventing the synthesis of proteins which are undesired or harmful to the cell/organism. This concept of oligonucleotide mediated
- 25 blocking of translation is known as the "antisense" approach. Mechanistically, the hybridising oligonucleotide is thought to elicit its effect by either creating a physical block to the translation process or by recruiting cellular enzymes that specifically degrades the mRNA part of the duplex (RNaseH).
- 30 More recently, oligoribonucleotides and oligodeoxyribonucleotides and analogues thereof which combine RNase catalytic activity with the ability to sequence specifically interact with a complementary RNA target (ribozymes) have attracted

much interest as antisense probes. Thus far ribozymes have been reported to be effective in cell cultures against both viral targets and oncogenes.

To completely prevent the synthesis of a given protein by the antisense approach it is
5 necessary to block/destroy all mRNAs that encode that particular protein and in many cases the number of these mRNA are fairly large. Typically, the mRNAs that encode a particular protein are transcribed from a single or a few genes. Hence, by targeting the gene ("antigene" approach) rather than its mRNA products it should be possible to either block production of its cognate protein more efficiently or to achieve a
10 significant reduction in the amount of oligonucleotides necessary to elicit the desired effect. To block transcription, the oligonucleotide must be able to hybridise sequence specifically to double stranded DNA. In 1953 Watson and Crick showed that deoxyribo nucleic acid (DNA) is composed of two strands (Nature, 1953, 171, 737) which are held together in a helical configuration by hydrogen bonds formed between
15 opposing complementary nucleobases in the two strands. The four nucleobases, commonly found in DNA are guanine (G), adenine (A), thymine (T) and cytosine (C) of which the G nucleobase pairs with C, and the A nucleobase pairs with T. In RNA the nucleobase thymine is replaced by the nucleobase uracil (U) which similarly to the T nucleobase pairs with A. The chemical groups in the nucleobases that participate in
20 standard duplex formation constitute the Watson-Crick face. In 1959, Hoogsteen showed that the purine nucleobases (G and A) in addition to their Watson-Crick face have a Hoogsteen face that can be recognised from the outside of a duplex, and used to bind pyrimidine oligonucleotides via hydrogen bonding, thereby forming a triple helix structure. Although making the "antigene" approach conceptually feasible the practical
25 usefulness of triple helix forming oligomers is currently limited by several factors including the requirement for homopurine sequence motifs in the target gene and a need for unphysiologically high ionic strength and low pH to stabilise the complex.

The use of oligonucleotides known as aptamers are also being actively investigated.
30 This promising new class of therapeutic oligonucleotides are selected *in vitro* to specifically bind to a given target with high affinity, such as for example ligand receptors. Their binding characteristics are likely a reflection of the ability of oligonucleotides to form three dimensional structures held together by intramolecular nucleobase pairing.

Likewise, nucleosides and nucleoside analogues have proven effective in chemotherapy of numerous viral infections and cancers.

Also, various types of double-stranded RNAs have been shown to effectively inhibit
5 the growth of several types of cancers.

Diagnostics

In molecular biology, oligonucleotides are routinely used for a variety of purposes such
10 as for example (i) as hybridisation probes in the capture, identification and quantification of target nucleic acids (ii) as affinity probes in the purification of target nucleic acids (iii) as primers in sequencing reactions and target amplification processes such as the polymerase chain reaction (PCR) (iv) to clone and mutate nucleic acids and (vi) as building blocks in the assembly of macromolecular structures.

15

Diagnostics utilises many of the oligonucleotide based techniques mentioned above in particular those that lend themselves to easy automation and facilitate reproducible results with high sensitivity. The objective in this field is to use oligonucleotide based techniques as a means to, for example (i) test humans, animals and food for the
20 presence of pathogenic micro-organisms (ii) to test for genetic predisposition to a disease (iii) to identify inherited and acquired genetic disorders, (iv) to link biological deposits to suspects in crime trials and (v) to validate the presence of micro-organisms involved in the production of foods and beverages.

25 General considerations

To be useful in the extensive range of different applications outlined above, oligonucleotides have to satisfy a large number of different requirements. In antisense therapeutics, for instance, a useful oligonucleotide must be able to penetrate the cell
30 membrane, have good resistance to extra- and intracellular nucleases and preferably have the ability to recruit endogenous enzymes like RNAseH. In DNA-based diagnostics and molecular biology other properties are important such as, e.g., the ability of oligonucleotides to act as efficient substrates for a wide range of different enzymes evolved to act on natural nucleic acids, such as e.g. polymerases, kinases,

- ligases and phosphatases. The fundamental property of oligonucleotides, however, which underlies all uses is their ability to recognise and hybridise sequence specifically to complementary single stranded nucleic acids employing either Watson-Crick hydrogen bonding (A-T and G-C) or other hydrogen bonding schemes such as the
- 5 Hoogsteen mode. The are two important terms affinity and specificity are commonly used to characterise the hybridisation properties of a given oligonucleotide. Affinity is a measure of the binding strength of the oligonucleotide to its complementary target sequence (expressed as the thermostability (T_m) of the duplex). Each nucleobase pair in the duplex adds to the thermostability and thus affinity increases with increasing
- 10 size (No. of nucleobases) of the oligonucleotide. Specificity is a measure of the ability of the oligonucleotide to discriminate between a fully complementary and a mismatched target sequence. In other words, specificity is a measure of the loss of affinity associated with mismatched nucleobase pairs in the target. At constant oligonucleotide size the specificity increases with increasing number of mismatches
- 15 between the oligonucleotide and its targets (*i.e.* the percentage of mismatches increases). Conversely, specificity decreases when the size of the oligonucleotide is increased at a constant number of mismatches (*i.e.* the percentage of mismatches decreases). Stated another way, an increase in the affinity of an oligonucleotide occurs at the expense of specificity and vice-versa.
- 20
- This property of oligonucleotides creates a number of problems for their practical use. In lengthy diagnostic procedures, for instance, the oligonucleotide needs to have both high affinity to secure adequate sensitivity of the test and high specificity to avoid false positive results. Likewise, an oligonucleotide used as antisense probes needs to
- 25 have both high affinity for its target mRNA to efficiently impair its translation and high specificity to avoid the unintentional blocking of the expression of other proteins. With enzymatic reactions, like, *e.g.*, PCR amplification, the affinity of the oligonucleotide primer must be high enough for the primer/target duplex to be stable in the temperature range where the enzymes exhibits activity, and specificity needs to be
- 30 high enough to ensure that only the correct target sequence is amplified.

Given the shortcomings of natural oligonucleotides, new approaches for enhancing specificity and affinity would be highly useful for DNA-based therapeutics, diagnostics and for molecular biology techniques in general.

Conformationally restricted nucleosides

It is known that oligonucleotides undergo a conformational transition in the course of
5 hybridising to a target sequence, from the relatively random coil structure of the single
stranded state to the ordered structure of the duplex state.

A number of conformationally restricted oligonucleotides including bicyclic and
tricyclic nucleoside analogues (Figure 1A and 1B in which B=nucleobase) have been
10 synthesised, incorporated into oligonucleotide and oligonucleotide analogues and
tested for their hybridisation and other properties.

Bicyclo[3.3.0] nucleosides (bcDNA) with an additional C-3',C-5'-ethano-bridge (A and
B) have been synthesised with all five nucleobases (G, A, T, C and U) whereas (C) has
15 been synthesised only with T and A nucleobases (M. Tarköy, M. Bolli, B. Schweizer
and C. Leumann, *Helv. Chim. Acta*, 1993, **76**, 481; Tarköy and C. Leumann, *Angew.
Chem., Int. Ed. Engl.*, 1993, **32**, 1432; M. Egli, P. Lubini, M. Dobler and C. Leumann,
J. Am. Chem. Soc., 1993, **115**, 5855; M. Tarköy, M. Bolli and C. Leumann, *Helv.
Chim. Acta*, 1994, **77**, 716; M. Bolli and C. Leumann, *Angew. Chem., Int. Ed. Engl.*,
20 1995, **34**, 694; M. Bolli, P. Lubini and C. Leumann, *Helv. Chim. Acta*, 1995, **78**,
2077; J. C. Litten, C. Epple and C. Leumann, *Bioorg. Med. Chem. Lett.*, 1995, **5**,
1231; J. C. Litten and C. Leumann, *Helv. Chim. Acta*, 1996, **79**, 1129; M. Bolli, J. C.
Litten, R. Schültz and C. Leumann, *Chem. Biol.*, 1996, **3**, 197; M. Bolli, H. U. Trafelet
and C. Leumann, *Nucleic Acids Res.*, 1996, **24**, 4660). DNA oligonucleotides
25 containing a few, or being entirely composed, of these analogues are in most cases
able to form Watson-Crick bonded duplexes with complementary DNA and RNA
oligonucleotides. The thermostability of the resulting duplexes, however, is either
distinctly lower (C), moderately lower (A) or comparable to (B) the stability of the
natural DNA and RNA counterparts. All bcDNA oligomers exhibited a pronounced
30 increase in sensitivity to the ionic strength of the hybridisation media compared to the
natural counterparts. The α -bicyclo-DNA (B) is more stable towards the 3'-
exonuclease snake venom phosphodiesterase than the β -bicyclo-DNA (A) which is
only moderately more stable than unmodified oligonucleotides.

- Bicarbocyclo[3.1.0]nucleosides with an additional C-1',C-6' or C-6',C-4'-methano-bridge on a cyclopentane ring (D and E, respectively) have been synthesised with all five nucleobases (T, A, G, C and U). Only the T-analogues, however, have been incorporated into oligomers. Incorporation of one or ten monomers D in a mixed poly-
- 5 pyrimidine DNA oligonucleotide resulted in a substantial decrease in the affinity towards both DNA and RNA oligonucleotides compared to the unmodified reference oligonucleotide. The decrease was more pronounced with ssDNA than with ssRNA. Incorporation of one monomer E in two different poly-pyrimidine DNA oligonucleotides induced modest increases in T_m 's of 0.8 °C and 2.1 °C for duplexes towards ssRNA
- 10 compared with unmodified reference duplexes. When ten T-analogues were incorporated into a 15mer oligonucleotide containing exclusively phosphorothioate internucleoside linkages, the T_m against the complementary RNA oligonucleotide was increased approximately 1.3 °C per modification compared to the same unmodified phosphorothioate sequence. Contrary to the control sequence the oligonucleotide
- 15 containing the bicyclic nucleoside E failed to mediate RNaseH cleavage. The hybridisation properties of oligonucleotides containing the G, A, C and U-analogues of E have not been reported. Also, the chemistry of this analogue does not lend itself to further intensive investigations on completely modified oligonucleotides (K.-H. Altmann, R. Kesselring, E. Francotte and G. Rihs, *Tetrahedron Lett.*, 1994, 35, 2331;
- 20 K.-H. Altmann, R. Imwinkelried, R. Kesselring and G. Rihs, *Tetrahedron Lett.*, 1994, 35, 7625; V. E. Marquez, M. A. Siddiqui, A. Ezzitouni, P. Russ, J. Wang, R. W. Wagner and M. D. Matteucci, *J. Med. Chem.*, 1996, 39, 3739; A. Ezzitouni and V. E. Marquez, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1073).
- 25 A bicyclo[3.3.0] nucleoside containing an additional C-2',C-3'-dioxalane ring has been synthesised as a dimer with an unmodified nucleoside where the additional ring is part of the internucleoside linkage replacing a natural phosphodiester linkage (F). This analogue was only synthesised as either thymine-thymine or thymine-5-methylcytosine blocks. A 15-mer polypyrimidine sequence containing seven of these
- 30 dimeric blocks and having alternating phosphodiester- and riboacetal-linkages, exhibited a substantially decreased T_m against complementary ssRNA compared to a control sequence with exclusively natural phosphodiester internucleoside linkages (R. J. Jones, S. Swaminathan, J. F. Milligan, S. Wadwani, B. S. Froehler and M. Matteucci, *J. Am. Chem. Soc.*, 1993, 115, 9816).

The two dimers (**G** and **H**) with additional C-2',C-3'-dioxane rings forming bicyclic[4.3.0]-systems in acetal-type internucleoside linkages have been synthesised as T-T dimers and incorporated once in the middle of 12mer polypyrimidine

- 5 oligonucleotides. Oligonucleotides containing either **G** or **H** both formed significantly less stable duplexes with complementary ssRNA and ssDNA compared with the unmodified control oligonucleotide (J. Wang and M. D. Matteucci, *Bioorg. Med. Chem. Lett.*, 1997, 7, 229).
- 10 Dimers containing a bicyclo[3.1.0]nucleoside with a C-2',C-3'-methano bridge as part of amide- and sulfonamide-type (**I** and **J**) internucleoside linkages have been synthesised and incorporated into oligonucleotides. Oligonucleotides containing one or more of these analogues showed a significant reduction in T_m compared to unmodified natural oligonucleotide references (C. G. Yannopoulos, W. Q. Zhou, P. Nower, D. Peach, Y. S. Sanghvi and G. Just, *Synlett*, 1997, 378).
- 15

A trimer with formacetal internucleoside linkages and a bicyclo[3.3.0] glucose-derived nucleoside analogue in the middle (**K**) has been synthesised and connected to the 3'-end of an oligonucleotide. The T_m against complementary ssRNA was decreased by 4
20 °C, compared to a control sequence, and by 1.5 °C compared to a sequence containing two 2',5'-formacetal linkages in the 3'-end (C. G. Yannopoulos, W. Q. Zhou, P. Nower, D. Peach, Y. S. Sanghvi and G. Just, *Synlett*, 1997, 378).

Very recently oligomers composed of tricyclic nucleoside-analogues (**L**) have been
25 reported to show increased duplex stability compared to natural DNA (R. Steffens and C. Leumann (Poster SB-B4), *Chimia*, 1997, 51, 436).

Three bicyclic ([4.3.0] and [3.3.0]) nucleosides with an additional C-2',C-3'-connected six- (**M** and **N**) or five-membered ring (**O**) have been synthesised as the T-
30 analogues. The bicyclic nucleosides **M** and **N** have been incorporated once and twice into 14-mer oligo-T sequences. The T_m 's against complementary ssRNA and ssDNA were decreased by 6-10 °C per modification compared to unmodified control sequences. Fully modified oligonucleotides of analogue **O** exhibited an increased T_m of approximately 1.0 °C per modification against the complementary RNA oligonucleotide

compared to the control DNA oligonucleotide. Also, the fully modified sequence was substantially more stable towards snake-venom phosphodiesterase hydrolysis than the unmodified control sequence. Partly modified oligonucleotides in which up to four analogues of O were incorporated, however, were less thermostable than the

5 corresponding unmodified oligonucleotides. All oligonucleotides containing analogue O (both fully and partly modified) showed a substantial decrease in thermostability against complementary DNA oligonucleotides compared to the unmodified oligonucleotides (P. Nielsen, H. M. Pfundheller, J. Wengel, *Chem. Commun.*, 1997, 826; P. Nielsen, H. M. Pfundheller, J. Wengel, XII International Roundtable:

10 Nucleosides, Nucleotides and Their Biological Applications; La Jolla, California, September 15-19, 1996; Poster PPI 43).

An attempt to make the bicyclic uridine nucleoside analogue Q planned to contain an additional O-2',C-4'-five-membered ring, starting from 4'-C-hydroxymethyl nucleoside

15 P, failed (K. D. Nielsen, *Specialerapport (Odense University, Denmark)*, 1995).

Until now the pursuit of conformationally restricted nucleosides useful in the formation of synthetic oligonucleotides with significantly improved hybridisation characteristics has met with little success. In the majority of cases, oligonucleotides containing these

20 analogues form less stable duplexes with complementary nucleic acids compared to the unmodified oligonucleotides. In other cases, where moderate improvement in duplex stability is observed, this relates only to either a DNA or an RNA target, or it relates to fully but not partly modified oligonucleotides or vice versa. An appraisal of most of the reported analogues are further complicated by the lack of data on

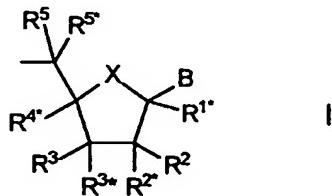
25 analogues with G, A and C nucleobases and lack of data indicating the specificity and mode of hybridisation. In many cases, synthesis of the reported monomer analogues is very complex while in other cases the synthesis of fully modified oligonucleotides is incompatible with the widely used phosphoramidite chemistry standard.

30 SUMMARY OF THE INVENTION

In view of the shortcomings of the previously known nucleoside analogues, the present inventors have now provided novel nucleoside analogues (LNAs) and oligonucleotides have included LNA nucleoside analogues therein. The novel LNA

nucleoside analogues have been provided with all commonly used nucleobases thereby providing a full set of nucleoside analogues for incorporation in oligonucleotides. As will be apparent from the following, the LNA nucleoside analogues and the LNA modified oligonucleotide provides a wide range of improvements for oligonucleotides
 5 used in the fields of diagnostics and therapy. Furthermore, the LNA nucleoside analogues and the LNA modified oligonucleotide also provides completely new perspectives in nucleoside and oligonucleotide based diagnostics and therapy.

Thus, the present invention relates to oligomers comprising at least one nucleoside
 10 analogue (hereinafter termed "LNA") of the general formula I



wherein X is selected from -O-, -S-, -N(R^{N*})-, -C(R⁶R^{6*})-, -O-C(R⁷R^{7*})-, -C(R⁶R^{6*})-O-, -S-C(R⁷R^{7*})-, -C(R⁶R^{6*})-S-, -N(R^{N*})-C(R⁷R^{7*})-, -C(R⁶R^{6*})-N(R^{N*})-, and -C(R⁶R^{6*})-C(R⁷R^{7*})-;

15 B is selected from hydrogen, hydroxy, optionally substituted C₁₋₄-alkoxy, optionally substituted C₁₋₄-alkyl, optionally substituted C₁₋₄-acyloxy, nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands;

20 P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R⁵;

one of the substituents R², R^{2*}, R³, and R^{3*} is a group P* which designates an
 25 internucleoside linkage to a preceding monomer, or a 3'-terminal group;

one or two pairs of non-geminal substituents selected from the present substituents of R^{1'}, R^{4'}, R⁵, R^{5*}, R⁶, R^{6*}, R⁷, R^{7*}, R^{N*}, and the ones of R², R^{2*}, R³, and R^{3*} not

designating P' each designates a biradical consisting of 1-8 groups/atoms selected from -C(R^aR^b)-, -C(R^a)=C(R^a)-, -C(R^a)=N-, -O-, -Si(R^a)₂-, -S-, -SO₂-, -N(R^a)-, and >C=Z, wherein Z is selected from -O-, -S-, and -N(R^a)-, and R^a and R^b each is independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl,

5 optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl, hydroxy, C₁₋₁₂-alkoxy, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl,

10 C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may

15 be optionally substituted, and where two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH₂), and wherein two non-geminal or geminal substituents selected from R^a, R^b, and any of the substituents R^{1*}, R², R^{2*}, R³, R^{3*}, R^{4*}, R⁵, R^{5*}, R⁶ and R^{6*}, R⁷, and R^{7*} which are present and not involved in P, P' or the biradical(s) together may form an

20 associated biradical selected from biradicals of the same kind as defined before; said pair(s) of non-geminal substituents thereby forming a mono- or bicyclic entity together with (i) the atoms to which said non-geminal substituents are bound and (ii) any intervening atoms; and

25 each of the substituents R^{1*}, R², R^{2*}, R³, R^{4*}, R⁵, R^{5*}, R⁶ and R^{6*}, R⁷, and R^{7*} which are present and not involved in P, P' or the biradical(s), is independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl, optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl, hydroxy, C₁₋₁₂-alkoxy, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy,

30 arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, DNA intercalators,

photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradical consisting of a

- 5 1-5 carbon atom(s) alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)- where R^N is selected from hydrogen and C₁₋₄-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C₁₋₄-alkyl;

10

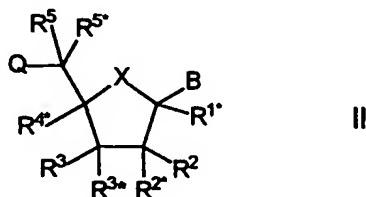
and basic salts and acid addition salts thereof;

with the proviso that,

- 15 (i) R² and R³ do not together designate a biradical selected from -O-CH₂-CH₂- and -O-CH₂-CH₂-CH₂- when LNA is a bicyclic nucleoside analogue;
- (ii) R³ and R⁵ do not together designate a biradical selected from -CH₂-CH₂-, -O-CH₂-, when LNA is a bicyclic nucleoside analogue;
- (iii) R³, R⁵, and R^{5*} do not together designate a triradical -CH₂-CH(-)-CH₂- when
20 LNA is a tricyclic nucleoside analogue;
- (iv) R^{1*} and R^{6*} do not together designate a biradical -CH₂- when LNA is a bicyclic nucleoside analogue; and
- (v) R^{4*} and R^{6*} do not together designate a biradical -CH₂- when LNA is a bicyclic nucleoside analogue.

25

The present invention furthermore relates to nucleoside analogues (hereinafter LNAs) of the general formula II



wherein the substituent B is selected from nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands;

5 X is selected from -O-, -S-, -N(R^{N*})-, and -C(R⁸R^{6*})-;

one of the substituents R², R^{2*}, R³, and R^{3*} is a group Q*;

each of Q and Q' is independently selected from hydrogen, azido, halogen, cyano,

- 10 nitro, hydroxy, Prot-O-, Act-O-, mercapto, Prot-S-, Act-S-, C₁₋₆-alkylthio, amino, Prot-N(R^H)-, Act-N(R^H)-, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkenyloxy, optionally substituted C₂₋₆-alkynyl, optionally substituted C₂₋₆-alkynyloxy, monophosphate, diphosphate, triphosphate, DNA intercalators,
- 15 photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphono, hydroxymethyl, Prot-O-CH₂-, Act-O-CH₂-, aminomethyl, Prot-N(R^H)-CH₂-, Act-N(R^H)-CH₂-, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and
- 20 C₁₋₆-alkyl;

- (i) R^{2*} and R^{4*} together designate a biradical selected from -O-, -(CR'R')_{r+s+1-}, -(CR'R')_r-O-(CR'R')_s-, -(CR'R')_r-S-(CR'R')_s-, -(CR'R')_r-N(R')-(CR'R')_s-, -O-(CR'R')_{r+s}-O-, -S-(CR'R')_{r+s}-O-, -O-(CR'R')_{r+s}-S-, -N(R')-(CR'R')_{r+s}-O-, -O-(CR'R')_{r+s}-N(R')-, -S-(CR'R')_{r+s}-S-, -N(R')-(CR'R')_{r+s}-N(R')-, -N(R')-(CR'R')_{r+s}-S-, and -S-(CR'R')_{r+s}-N(R')-;
- (ii) R² and R³ together designate a biradical selected from -O-, -(CR'R')_{r+s}-, -(CR'R')_r-O-(CR'R')_s-, -(CR'R')_r-S-(CR'R')_s-, and -(CR'R')_r-N(R')-(CR'R')_s-;
- (iii) R^{2*} and R³ together designate a biradical selected from -O-, -(CR'R')_{r+s}-, -(CR'R')_r-O-(CR'R')_s-, -(CR'R')_r-S-(CR'R')_s-, and -(CR'R')_r-N(R')-(CR'R')_s-;
- (iv) R³ and R^{4*} together designate a biradical selected from -(CR'R')_r-O-(CR'R')_s-, -(CR'R')_r-S-(CR'R')_s-, and -(CR'R')_r-N(R')-(CR'R')_s-;
- (v) R³ and R⁵ together designate a biradical selected from -(CR'R')_r-O-(CR'R')_s-, -(CR'R')_r-S-(CR'R')_s-, and -(CR'R')_r-N(R')-(CR'R')_s-; or

- (vi) R^{1*} and R^{4*} together designate a biradical selected from -(CR^{*}R^{*}),-O-(CR^{*}R^{*}),-, -(CR^{*}R^{*}),-S-(CR^{*}R^{*}),-, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*}),-;
- (vii) R^{1*} and R^{2*} together designate a biradical selected from -(CR^{*}R^{*}),-O-(CR^{*}R^{*}),-, -(CR^{*}R^{*}),-S-(CR^{*}R^{*}),-, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*}),-;

5

wherein each R^{*} is independently selected from hydrogen, halogen, azido, cyano, nitro, hydroxy, mercapto, amino, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and/or two adjacent (non-geminal) R^{*} may together designate a double bond, and each of r and s is 0-3 with the proviso that the sum r+s is 1-4;

- each of the substituents R^{1*}, R², R^{2*}, R³, R^{4*}, R⁵, and R^{5*}, which are not involved in Q,
- 15 Q^{*} or the biradical, is independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl, optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl, hydroxy, C₁₋₁₂-alkoxy, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and
- 20 ligands, where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradical consisting of a 1-5 carbon atom(s) alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)- where R^N is selected
- 25 from hydrogen and C₁₋₄-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C₁₋₄-alkyl;

and basic salts and acid addition salts thereof;

with the first proviso that,

- 5 (i) R^2 and R^3 do not together designate a biradical selected from $-O-CH_2-CH_2-$ and $-O-CH_2-CH_2-CH_2-$; and
- (ii) R^3 and R^5 do not together designate a biradical selected from $-CH_2-CH_2-$,
 $-O-CH_2-$, and $-O-Si(iPr)_2-O-Si(iPr)_2-O-$;

and with the second proviso that any chemical group (including any nucleobase),

10 which is reactive under the conditions prevailing in oligonucleotide synthesis, is
optionally functional group protected.

The present invention also relates to the use of the nucleoside analogues (LNAs) for
the preparation of oligomers, and the use of the oligomers as well as the nucleoside
15 analogues (LNAs) in diagnostics, molecular biology research, and in therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B illustrate known conformationally restricted nucleotides.

20

Figure 2 illustrates nucleotide/nucleoside analogues of the invention.

Figure 3 illustrates the performance of LNA modified oligonucleotides in the sequence
specific capture of PCR amplicons.

25

Figures 4A and 4B illustrate that LNA modified oligonucleotides are able to capture its
cognate PCR amplicon by strand invasion.

Figure 5 illustrates that LNA modified oligonucleotides, immobilised on a solid surface,
30 function efficiently in the sequence specific capture of a PCR amplicon.

Figure 6 illustrates that LNA modified oligonucleotides can act as substrates for T4
polynucleotide kinase.

Figure 7 illustrates that LNA modified oligonucleotides can function as primers for nucleic acid polymerases.

Figure 8 illustrates that LNA modified oligonucleotides can functions as primers in
5 target amplification processes.

Figure 9 illustrates that LNA modified oligonucleotides carrying a 5' anthraquinone can be covalently immobilised on a solid support by irradiation and that the immobilised oligomer is efficient in the capture of a complementary DNA oligo.

10

Figure 10 illustrates that LNA-thymidine-5'-triphosphate (LNA-TTP) can act as a substrate for terminal deoxynucleotidyl transferase (TdT).

15

Figure 11 illustrates hybridisation and detection on an array with different LNA
15 modified Cy3-labelled 8mers.

Figures 12 and 13 illustrate hybridisation and detection of end mismatches on an array with LNA modified Cy3-labelled 8mers.

20

Figure 14 illustrates blockade by LNA of [D-Ala2]deltorphin-induced antinociception in the warm water tail flick test in conscious rats.

Figures 15A, 15B, and 15C illustrate Hybridization and detection of end mismatches on an array with AT and all LNA modified Cy3-labelled 8mers.

25

Figures 16 and 17 illustrate that LNA can be delivered to living human MCF-7 breast cancer cells.

30

Figures 18 and 19 illustrate the use of [$\alpha^{33}\text{P}$] ddNTP's and ThermoSequenaseTM DNA Polymerase to sequence DNA templates containing LNA T monomers.

Figures 20 and 21 illustrate that exonuclease free Klenow fragment DNA polymerase I can incorporate LNA Adenosine, Cytosine, Guanosine and Uridine-5'-triphosphates into a DNA strand.

Figure 22 illustrates the ability of terminal deoxynucleotidyl transferase (TdT) to tail LNA modified oligonucleotides.

- 5 Figures 23A and 23B illustrate that fully mixed LNA monomers can be used to significantly increase the performance of immobilised biotinylated-DNA oligos in the sequence specific capture of PCR amplicons.

Figures 24 to 41 illustrates possible synthetic routes towards the LNA monomers of
10 the invention.

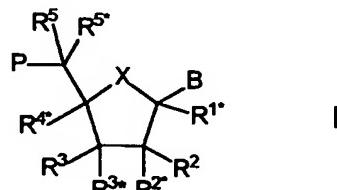
DETAILED DESCRIPTION OF THE INVENTION

When used herein, the term "**LNA**" (Locked Nucleoside Analogues) refers to the bi-
15 and tri-cyclic nucleoside analogues of the invention, either incorporated in the oligomer of the invention (general formula I) or as discrete chemical species (general formula II). The term "monomeric LNA" specifically refers to the latter case.

Oligomers and nucleoside analogues

20 As mentioned above, the present invention *i.a.* relates to novel oligomers (oligonucleotides) comprising one or more bi-, tri-, or polycyclic nucleoside analogues (hereinafter termed "**LNA**"). It has been found that the incorporation of such LNAs in place of, or as a supplement to, e.g., known nucleosides confer interesting and highly 25 useful properties to an oligonucleotide. Bi- and tricyclic, especially bicyclic, LNAs seem especially interesting within the scope of the present invention.

Each of the possible LNAs incorporated in an oligomer (oligonucleotide) has the general formula I



wherein X is selected from -O- (the furanose motif), -S-, -N(R^{N*})-, -C(R⁶R^{6*})-, -O-C(R⁷R^{7*})-, -C(R⁶R^{6*})-O-, -S-C(R⁷R^{7*})-, -C(R⁶R^{6*})-S-, -N(R^{N*})-C(R⁷R^{7*})-, -C(R⁶R^{6*})-N(R^{N*})-, and -C(R⁶R^{6*})-C(R⁷R^{7*})-, where R⁶, R^{6*}, R⁷, R^{7*}, and R^{N*} are as defined further below.

- 5 Thus, the LNAs incorporated in the oligomer may comprise an either 5- or 6-membered ring as an essential part of the bi-, tri-, or polycyclic structure. It is believed that 5-membered rings (X = -O-, -S-, -N(R^{N*})-, -C(R⁶R^{6*})-) are especially interesting in that they are able to occupy essentially the same conformations (however locked by the introduction of one or more biradicals (see below)) as the native furanose ring of a
10 naturally occurring nucleoside. Among the possible 5-membered rings, the situations where X designates -O-, -S-, and -N(R^{N*})- seem especially interesting, and the situation where X is -O- appears to be particularly interesting.

- The substituent B may designate a group which, when the oligomer is complexing
15 with DNA or RNA, is able to interact (e.g. by hydrogen bonding or covalent bonding or electronic interaction) with DNA or RNA, especially nucleobases of DNA or RNA. Alternatively, the substituent B may designate a group which acts as a label or a reporter, or the substituent B may designate a group (e.g. hydrogen) which is expected to have little or no interactions with DNA or RNA. Thus, the substituent B is
20 preferably selected from hydrogen, hydroxy, optionally substituted C₁₋₄-alkoxy, optionally substituted C₁₋₄-alkyl, optionally substituted C₁₋₄-acyloxy, nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands.

- 25 In the present context, the terms "nucleobase" covers naturally occurring nucleobases as well as non-naturally occurring nucleobases. It should be clear to the person skilled in the art that various nucleobases which previously have been considered "non-naturally occurring" have subsequently been found in nature. Thus, "nucleobase" includes not only the known purine and pyrimidine heterocycles, but also heterocyclic
30 analogues and tautomers thereof. Illustrative examples of nucleobases are adenine, guanine, thymine, cytosine, uracil, purine, xanthine, diaminopurine, 8-oxo-N⁶-methyladenine, 7-deazaxanthine, 7-deazaguanine, N⁴,N⁴-ethanocytosin, N⁶,N⁶-ethano-2,6-diaminopurine, 5-methylcytosine, 5-(C³-C⁶)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudouracil, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine,

isoguanin, inosine and the "non-naturally occurring" nucleobases described in Benner et al., U.S. Pat No. 5,432,272. The term "nucleobase" is intended to cover every and all of these examples as well as analogues and tautomers thereof. Especially interesting nucleobases are adenine, guanine, thymine, cytosine, and uracil, which are 5 considered as the naturally occurring nucleobases in relation to therapeutic and diagnostic application in humans.

When used herein, the term "DNA intercalator" means a group which can intercalate into a DNA or RNA helix, duplex or triplex. Examples of functional parts of DNA 10 intercalators are acridines, anthracene, quinones such as anthraquinone, indole, quinoline, isoquinoline, dihydroquinones, anthracyclines, tetracyclines, methylene blue, anthracyclinone, psoralens, coumarins, ethidium-halides, dynemicin, metal complexes such as 1,10-phenanthroline-copper, tris(4,7-diphenyl-1,10-phenanthroline)ruthenium-cobalt-enediynes such as calicheamicin, porphyrins, distamycin, netropsin, viologen, 15 daunomycin. Especially interesting examples are acridines, quinones such as anthraquinone, methylene blue, psoralens, coumarins, and ethidium-halides.

In the present context, the term "photochemically active groups" covers compounds which are able to undergo chemical reactions upon irradiation with light. Illustrative 20 examples of functional groups hereof are quinones, especially 6-methyl-1,4-naphtoquinone, anthraquinone, naphtoquinone, and 1,4-dimethyl-antraquinone, diazirines, aromatic azides, benzophenones, psoralens, diazo compounds, and diazirino compounds.

25 In the present context "thermochemically reactive group" is defined as a functional group which is able to undergo thermochemically-induced covalent bond formation with other groups. Illustrative examples of functional parts thermochemically reactive groups are carboxylic acids, carboxylic acid esters such as activated esters, carboxylic acid halides such as acid fluorides, acid chlorides, acid bromide, and acid iodides, 30 carboxylic acid azides, carboxylic acid hydrazides, sulfonic acids, sulfonic acid esters, sulfonic acid halides, semicarbazides, thiosemicarbazides, aldehydes, ketones, primary alkohols, secondary alkohols, tertiary alkohols, phenols, alkyl halides, thiols, disulphides, primary amines, secondary amines, tertiary amines, hydrazines, epoxides, maleimides, and boronic acid derivatives.

In the present context, the term "chelating group" means a molecule that contains more than one binding site and frequently binds to another molecule, atom or ion through more than one binding site at the same time. Examples of functional parts of 5 chelating groups are iminodiacetic acid, nitrilotriacetic acid, ethylenediamine tetraacetic acid (EDTA), aminophosphonic acid, etc.

In the present context, the term "reporter group" means a group which is detectable either by itself or as a part of an detection series. Examples of functional parts of 10 reporter groups are biotin, digoxigenin, fluorescent groups (groups which are able to absorb electromagnetic radiation, e.g. light or X-rays, of a certain wavelength, and which subsequently reemits the energy absorbed as radiation of longer wavelength; illustrative examples are dansyl (5-dimethylamino)-1-naphthalenesulfonyl), DOXYL (N-oxy-4,4-dimethyloxazolidine), PROXYL (N-oxy-2,2,5,5-tetramethylpyrrolidine), 15 TEMPO (N-oxy-2,2,6,6-tetramethylpiperidine), dinitrophenyl, acridines, coumarins, Cy3 and Cy5 (trademarks for Biological Detection Systems, Inc.), erytrosine, coumaric acid, umbelliferone, texas red, rhodamine, tetramethyl rhodamine, Rox, 7-nitrobenzo-2-oxa-1-diazole (NBD), pyrene, fluorescein, Europium, Ruthenium, Samarium, and other rare earth metals), radioisotopic labels, chemiluminescence labels (labels that are 20 detectable via the emission of light during a chemical reaction), spin labels (a free radical (e.g. substituted organic nitroxides) or other paramagnetic probes (e.g. Cu²⁺, Mg²⁺) bound to a biological molecule being detectable by the use of electron spin resonance spectroscopy), enzymes (such as peroxidases, alkaline phosphatases, β-galactosidases, and glycose oxidases), antigens, antibodies, haptens (groups which 25 are able to combine with an antibody, but which cannot initiate an immune response by itself, such as peptides and steroid hormones), carrier systems for cell membrane penetration such as: fatty acid residues, steroid moieties (cholesteryl), vitamin A, vitamin D, vitamin E, folic acid peptides for specific receptors, groups for mediating endocytose, epidermal growth factor (EGF), bradykinin, and platelet derived growth 30 factor (PDGF). Especially interesting examples are biotin, fluorescein, Texas Red, rhodamine, dinitrophenyl, digoxigenin, Ruthenium, Europium, Cy5, Cy3, etc.

In the present context "ligand" means something which binds. Ligands can comprise functional groups such as: aromatic groups (such as benzene, pyridine, naphtalene,

- anthracene, and phenanthrene), heteroaromatic groups (such as thiophene, furan, tetrahydrofuran, pyridine, dioxane, and pyrimidine), carboxylic acids, carboxylic acid esters, carboxylic acid halides, carboxylic acid azides, carboxylic acid hydrazides, sulfonic acids, sulfonic acid esters, sulfonic acid halides, semicarbazides,
- 5 thiosemicarbazides, aldehydes, ketones, primary alcohols, secondary alcohols, tertiary alcohols, phenols, alkyl halides, thiols, disulphides, primary amines, secondary amines, tertiary amines, hydrazines, epoxides, maleimides, C₁-C₂₀ alkyl groups optionally interrupted or terminated with one or more heteroatoms such as oxygen atoms, nitrogen atoms, and/or sulphur atoms, optionally containing aromatic or
- 10 mono/polyunsaturated hydrocarbons, polyoxyethylene such as polyethylene glycol, oligo/polyamides such as poly-β-alanine, polyglycine, polylysine, peptides, oligo/polysaccharides, oligo/polyphosphates, toxins, antibiotics, cell poisons, and steroids, and also "affinity ligands", *i.e.* functional groups or biomolecules that have a specific affinity for sites on particular proteins, antibodies, poly- and oligosaccharides,
- 15 and other biomolecules.

It will be clear for the person skilled in the art that the above-mentioned specific examples under DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands correspond to the

20 "active/functional" part of the groups in question. For the person skilled in the art it is furthermore clear that DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands are typically represented in the form M-K- where M is the "active/functional" part of the group in question and where K is a spacer through which the "active/functional" part

25 is attached to the 5- or 6-membered ring. Thus, it should be understood that the group B, in the case where B is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, has the form M-K-, where M is the "active/functional" part of the DNA intercalator, photochemically active group, thermochemically active group, chelating

30 group, reporter group, and ligand, respectively, and where K is an optional spacer comprising 1-50 atoms, preferably 1-30 atoms, in particular 1-15 atoms, between the 5- or 6-membered ring and the "active/functional" part.

In the present context, the term "spacer" means a thermochemically and photochemically non-active distance-making group and is used to join two or more different moieties of the types defined above. Spacers are selected on the basis of a variety of characteristics including their hydrophobicity, hydrophilicity, molecular flexibility and length (e.g. see Hermanson et. al., "Immobilized Affinity Ligand Techniques", Academic Press, San Diego, California (1992), p. 137-ff). Generally, the length of the spacers are less than or about 400 Å, in some applications preferably less than 100 Å. The spacer, thus, comprises a chain of carbon atoms optionally interrupted or terminated with one or more heteroatoms, such as oxygen atoms, nitrogen atoms, and/or sulphur atoms. Thus, the spacer K may comprise one or more amide, ester, amino, ether, and/or thioether functionalities, and optionally aromatic or mono/polyunsaturated hydrocarbons, polyoxyethylene such as polyethylene glycol, oligo/polyamides such as poly-β-alanine, polyglycine, polylysine, and peptides in general, oligosaccharides, oligo/polyphosphates. Moreover the spacer may consist of combined units thereof. The length of the spacer may vary, taking into consideration the desired or necessary positioning and spatial orientation of the "active/functional" part of the group in question in relation to the 5- or 6-membered ring. In particularly interesting embodiments, the spacer includes a chemically cleavable group. Examples of such chemically cleavable groups include disulphide groups cleavable under reductive conditions, peptide fragments cleavable by peptidases, etc.

In one embodiment of the present invention, K designates a single bond so that the "active/functional" part of the group in question is attached directly to the 5- or 6-membered ring.

- 25
- In a preferred embodiment, the substituent B in the general formulae I and II is preferably selected from nucleobases, in particular from adenine, guanine, thymine, cytosine and urasil.
- 30 In the oligomers of the present invention (formula I), P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group. The first possibility applies when the LNA in question is not the 5'-terminal "monomer", whereas the latter possibility applies when the LNA in question is the 5'-terminal "monomer". It should be understood (which also will be clear from the definition of

internucleoside linkage and 5'-terminal group further below) that such an internucleoside linkage or 5'-terminal group may include the substituent R⁵ (or equally applicable: the substituent R^{5'}) thereby forming a double bond to the group P. (5'-Terminal refers to the position corresponding to the 5' carbon atom of a ribose moiety 5 in a nucleoside.)

On the other hand, an internucleoside linkage to a preceding monomer or a 3'-terminal group (P') may originate from the positions defined by one of the substituents R², R^{2'}, R³, and R^{3'}, preferably from the positions defined by one of the substituents R³ and 10 R^{3'}. Analogously, the first possibility applies where the LNA in question is not the 3'-terminal "monomer", whereas the latter possibility applies when the LNA in question is the 3'-terminal "monomer". (3'-Terminal refers to the position corresponding to the 3' carbon atom of a ribose moiety in a nucleoside.)

15 In the present context, the term "monomer" relates to naturally occurring nucleosides, non-naturally occurring nucleosides, PNAs, etc. as well as LNAs. Thus, the term "succeeding monomer" relates to the neighbouring monomer in the 5'-terminal direction and the "preceding monomer" relates to the neighbouring monomer in the 3'-terminal direction. Such succeeding and preceding monomers, seen from the position 20 of an LNA monomer, may be naturally occurring nucleosides or non-naturally occurring nucleosides, or even further LNA monomers.

Consequently, in the present context (as can be derived from the definitions above), the term "oligomer" means an oligonucleotide modified by the incorporation of one or 25 more LNA(s).

The crucial part of the present invention is the presence of one or more rings fused to the 5- or 6-membered ring illustrated with the general formula I. Thus, one or two pairs of non-geminal substituents selected from the present substituents of R^{1'}, R^{4'}, 30 R⁵, R^{5'}, R⁶, R^{6'}, R⁷, R^{7'}, R^{N'}, and the ones of R², R^{2'}, R³, and R^{3'} not designating P' each designates a biradical consisting of 1-8 groups/atoms, preferably 1-4 groups/atoms, independently selected from -C(R^aR^b)-, -C(R^a)=C(R^a)-, -C(R^a)=N-, -O-, -Si(R^a)₂-, -S-, -SO₂-, -N(R^a)-, and >C=Z. (The term "present" indicates that the

existence of some of the substituents, i.e. R⁸, R^{8*}, R⁷, R^{7*}, R^{N*}, is dependent on whether X includes such substituents.)

In the groups constituting the biradical(s), Z is selected from -O-, -S-, and -N(R^a)-, and

- 5 R^a and R^b each is independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl, optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl, hydroxy, C₁₋₁₂-alkoxy, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphonyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands (where
- 10 the latter groups may include a spacer as defined for the substituent B), where aryl and heteroaryl may be optionally substituted. Moreover, two geminal substituents R^a and R^b together may designate optionally substituted methylene (=CH₂ optionally substituted one or two times with substituents as defined as optional substituents for aryl), and two non-geminal or geminal substituents selected from R^a, R^b, and any of
- 15 the substituents R^{1*}, R², R^{2*}, R³, R^{3*}, R^{4*}, R⁵, R^{5*}, R⁶ and R^{6*}, R⁷, and R^{7*} which are present and not involved in P, P^{*} or the biradical(s) may together form an associated biradical selected from biradicals of the same kind as defined before. It will be clear that each of the pair(s) of non-geminal substituents thereby forms a mono- or bicyclic entity together with (i) the atoms to which the non-geminal substituents are bound
- 20 and (ii) any intervening atoms.
- 25

It is believed that biradicals which are bound to the ring atoms of the 5- or 6-membered rings are preferred in that inclusion of the substituents R⁵ and R^{5*} may cause an undesired sterical interaction with internucleoside linkage. Thus, it is

- 30 preferred that the one or two pairs of non-geminal substituents, which are constituting one or two biradical(s), respectively, are selected from the present substituents of R^{1*}, R^{4*}, R⁶, R^{8*}, R⁷, R^{7*}, R^{N*}, and the ones of R², R^{2*}, R³, and R^{3*} not designating P^{*}.

Preferably, the LNAs incorporated in the oligomers comprise only one biradical constituted by a pair of (two) non-geminal substituents. In particular, it is preferred that R^{3*} designates P* and that the biradical is formed between R^{2*} and R^{4*} or R² and R³.

5

This being said, it should be understood (especially with due consideration of the known bi- and tricyclic nucleoside analogues - see "Background of the Invention") that the present invention does not relate to oligomers comprising the following bi- or tricyclic nucleosides analogues:

10

- (i) R² and R³ together designate a biradical selected from -O-CH₂-CH₂- and -O-CH₂-CH₂-CH₂- when LNA is a bicyclic nucleoside analogue;
- (ii) R³ and R⁵ together designate a biradical selected from -CH₂-CH₂-, -O-CH₂-, when LNA is a bicyclic nucleoside analogue;
- 15 (iii) R³, R⁵, and R^{5*} together designate a triradical -CH₂-CH(-)-CH₂- when LNA is a tricyclic nucleoside analogue;
- (iv) R^{1*} and R^{6*} together designate a biradical -CH₂- when LNA is a bicyclic nucleoside analogue; or
- (v) R^{4*} and R^{6*} together designate a biradical -CH₂- when LNA is a bicyclic nucleoside analogue;

20 except where such bi- or tricyclic nucleoside analogues are combined with one or more of the novel LNAs defined herein.

- 25 In the present context, *i.e.* in the present description and claims, the orientation of the biradicals are so that the left-hand side represents the substituent with the lowest number and the right-hand side represents the substituent with the highest number, thus, when R³ and R⁵ together designate a biradical "-O-CH₂-", it is understood that the oxygen atom represents R³, thus the oxygen atom is *e.g.* attached to the position 30 of R³, and the methylene group represents R⁵.

Considering the numerous interesting possibilities for the structure of the biradical(s) in LNA(s) incorporated in oligomers according to the invention, it is believed that the biradical(s) constituted by pair(s) of non-geminal substituents preferably is/are selected

- from $-(CR^{\bullet}R^{\bullet})_r-Y-(CR^{\bullet}R^{\bullet})_{s-}$, $-(CR^{\bullet}R^{\bullet})_r-Y-(CR^{\bullet}R^{\bullet})_{s-}Y-$, $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$, $-Y-(CR^{\bullet}R^{\bullet})_rY-(CR^{\bullet}R^{\bullet})_{s-}$, $-(CR^{\bullet}R^{\bullet})_{r+s-}Y-$, $-Y-Y-$, wherein each Y is independently selected from -O-, -S-, -Si(R¹)₂-, -N(R¹)-, >C=O, -C(=O)-N(R¹)-, and -N(R¹)-C(=O)-, each R¹ is independently selected from hydrogen, halogen, azido, cyano, nitro, hydroxy,
- 5 mercapto, amino, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and/or two adjacent (non-geminal) R¹ may together designate a double bond; and each of r and s is 0-4 with the proviso that the sum r+s is 1-5. Particularly interesting
- 10 situations are those wherein each biradical is independently selected from -Y-, $-(CR^{\bullet}R^{\bullet})_{r+s-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$, wherein and each of r and s is 0-3 with the proviso that the sum r+s is 1-4.

- Considering the positioning of the biradical in the LNA(s), it is believed (based on the preliminary findings (see the examples)) that the following situations are especially interesting, namely where: R^{2*} and R^{4*} together designate a biradical selected from -Y-, $-(CR^{\bullet}R^{\bullet})_{r+s+1-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$; R² and R³ together designate a biradical selected from -Y-, $-(CR^{\bullet}R^{\bullet})_{r+s-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$; R^{2*} and R³ together designate a biradical selected from -Y-, $-(CR^{\bullet}R^{\bullet})_{r+s-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$;
- 20 R^{2*} and R^{4*} together designate a biradical selected from -Y-, $-(CR^{\bullet}R^{\bullet})_{r+s-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$; R³ and R⁵ together designate a biradical selected from -Y¹-, $-(CR^{\bullet}R^{\bullet})_{r+s+1-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$; R³ and R⁶ together designate a biradical selected from -Y¹-, $-(CR^{\bullet}R^{\bullet})_{r+s+1-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$; R^{1*} and R^{4*} together designate a biradical selected from -Y¹-, $-(CR^{\bullet}R^{\bullet})_{r+s+1-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}NR^{\bullet}-$; or where R^{1*} and R^{2*}
- 25 together designate a biradical selected from -Y-, $-(CR^{\bullet}R^{\bullet})_{r+s-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, and $-Y-(CR^{\bullet}R^{\bullet})_{r+s}Y-$; wherein each of r and s is 0-3 with the proviso that the sum r+s is 1-4, Y¹ is as defined above, and where Y¹ is selected from -NR¹-C(=O)- and -C(=O)-NR¹-.
- 30 Particularly interesting oligomers are those wherein one of the following criteria applies for at least one LNA in an oligomer: R^{2*} and R^{4*} together designate a biradical selected from -O-, -S-, -N(R¹)-, $-(CR^{\bullet}R^{\bullet})_{r+s+1-}Y-(CR^{\bullet}R^{\bullet})_{s-}$, $-(CR^{\bullet}R^{\bullet})_rO-(CR^{\bullet}R^{\bullet})_{s-}$, $-(CR^{\bullet}R^{\bullet})_rS-(CR^{\bullet}R^{\bullet})_{s-}$, $-(CR^{\bullet}R^{\bullet})_rN(R^{\bullet})-(CR^{\bullet}R^{\bullet})_{s-}$, $-O-(CR^{\bullet}R^{\bullet})_{r+s}O-$, $-S-(CR^{\bullet}R^{\bullet})_{r+s}O-$, $-O-(CR^{\bullet}R^{\bullet})_{r+s}S-$, $-N(R^{\bullet})-(CR^{\bullet}R^{\bullet})_{r+s}O-$, $-O-(CR^{\bullet}R^{\bullet})_{r+s}N(R^{\bullet})-$, $-S-(CR^{\bullet}R^{\bullet})_{r+s}S-$, $-N(R^{\bullet})-(CR^{\bullet}R^{\bullet})_{r+s}N(R^{\bullet})-$, $-N(R^{\bullet})-(CR^{\bullet}R^{\bullet})_{r+s}O-$.

- (CR¹R²)_{r+s}-S-, and -S-(CR¹R²)_{r+s}-N(R³)-; R² and R³ together designate a biradical selected from -O-, -(CR¹R²)_{r+s}-, -(CR¹R²),-O-(CR¹R²)_r, -(CR¹R²),-S-(CR¹R²)_r, and -(CR¹R²),-N(R³)-(CR¹R²)_r; R^{2*} and R³ together designate a biradical selected from -O-, -(CR¹R²)_{r+s}-, -(CR¹R²),-O-(CR¹R²)_r, -(CR¹R²),-S-(CR¹R²)_r, and -(CR¹R²),-N(R³)-(CR¹R²)_r;
- 5 R³ and R^{4*} together designate a biradical selected from -(CR¹R²),-O-(CR¹R²)_r, -(CR¹R²),-S-(CR¹R²)_r, and -(CR¹R²),-N(R³)-(CR¹R²)_r; R³ and R⁵ together designate a biradical selected from -(CR¹R²),-O-(CR¹R²)_r, -(CR¹R²),-S-(CR¹R²)_r, and -(CR¹R²),-N(R³)-(CR¹R²)_r; R^{1*} and R^{4*} together designate a biradical selected from -(CR¹R²),-O-(CR¹R²)_r, -(CR¹R²),-S-(CR¹R²)_r, and -(CR¹R²),-N(R³)-(CR¹R²)_r; or R^{1*} and R^{2*} together designate a biradical selected from -(CR¹R²),-O-(CR¹R²)_r, -(CR¹R²),-S-(CR¹R²)_r, and -(CR¹R²),-N(R³)-(CR¹R²)_r;
- 10 selected from -(CR¹R²),-O-(CR¹R²)_r, -(CR¹R²),-S-(CR¹R²)_r, and -(CR¹R²),-N(R³)-(CR¹R²)_r; wherein each of r and s is 0-3 with the proviso that the sum r+s is 1-4, and where R^H designates hydrogen or C₁₋₄-alkyl.

- It is furthermore preferred that one R^{*} is selected from hydrogen, hydroxy, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and any remaining substituents R^{*} are hydrogen.

- In one preferred embodiment, one group R^{*} in the biradical of at least one LNA is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B).

- With respect to the substituents R^{1*}, R², R^{2*}, R³, R^{4*}, R⁵, R^{5*}, R⁶ and R^{6*}, R⁷, and R^{7*}, which are present and not involved in P, P^{*} or the biradical(s), these are independently selected from hydrogen, optionally substituted C₁₋₁₂-alkyl, optionally substituted C₂₋₁₂-alkenyl, optionally substituted C₂₋₁₂-alkynyl, hydroxy, C₁₋₁₂-alkoxy, C₂₋₁₂-alkenyloxy, carboxy, C₁₋₁₂-alkoxycarbonyl, C₁₋₁₂-alkylcarbonyl, formyl, aryl, aryloxy-carbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxy-carbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, azido, sulphanyl, C₁₋₆-alkylthio, halogen, DNA intercalators, photochemically active groups, thermochemically active groups,

- chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B), where aryl and heteroaryl may be optionally substituted, and where two geminal substituents together may designate oxo, thioxo, imino, or optionally substituted methylene, or together may form a spiro biradical
- 5 consisting of a 1-5 carbon atom(s) alkylene chain which is optionally interrupted and/or terminated by one or more heteroatoms/groups selected from -O-, -S-, and -(NR^N)- where R^N is selected from hydrogen and C₁₋₄-alkyl, and where two adjacent (non-geminal) substituents may designate an additional bond resulting in a double bond; and R^{N*}, when present and not involved in a biradical, is selected from hydrogen
- 10 and C₁₋₄-alkyl.

- Preferably, each of the substituents R^{1*}, R², R^{2*}, R³, R^{3*}, R^{4*}, R⁵, R^{5*}, R⁶, R^{6*}, R⁷, and R^{7*} of the LNA(s), which are present and not involved in P, P* or the biradical(s), is independently selected from hydrogen, optionally substituted C₁₋₆-alkyl, optionally
- 15 substituted C₂₋₆-alkenyl, hydroxy, C₁₋₆-alkoxy, C₂₋₆-alkenyloxy, carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, azido, C₁₋₆-alkanoyloxy, sulphono, sulphanyl, C₁₋₆-alkylthio, DNA intercalators, photochemically active groups, thermochemically active groups,
- 20 chelating groups, reporter groups, and ligands, and halogen, where two geminal substituents together may designate oxo, and where R^{N*}, when present and not involved in a biradical, is selected from hydrogen and C₁₋₄-alkyl.

- In a preferred embodiment of the present invention, X is selected from -O-, -S-, and
- 25 -NR^{N*}-, in particular -O-, and each of the substituents R^{1*}, R², R^{2*}, R³, R^{3*}, R^{4*}, R⁵, R^{5*}, R⁶, R^{6*}, R⁷, and R^{7*} of the LNA(s), which are present and not involved in P, P* or the biradical(s), designate hydrogen.

- In an even more preferred embodiment of the present invention, R^{2*} and R^{4*} of an LNA
- 30 incorporated into an oligomer together designate a biradical. Preferably, X is O, R² selected from hydrogen, hydroxy, and optionally substituted C₁₋₆-alkoxy, and R^{1*}, R³, R⁵, and R^{6*} designate hydrogen, and, more specifically, the biradical is selected from -O-, -(CH₂)₀₋₁-O-(CH₂)₁₋₃-, -(CH₂)₀₋₁-S-(CH₂)₁₋₃-, -(CH₂)₀₋₁-N(R^N)-(CH₂)₁₋₃-, and -(CH₂)₂₋₄-, in particular from -O-CH₂-, -S-CH₂-, and -NR^H-CH₂-. Generally, with due regard to the

results obtained so far, it is preferred that the biradical constituting R^{2*} and R^{4*} forms a two carbon atom bridge, i.e. the biradical forms a five membered ring with the furanose ring (X = O).

- 5 In another embodiment of the present invention, R² and R³ of an LNA incorporated into an oligomer together designate a biradical. Preferably, X is O, R^{2*} is selected from hydrogen, hydroxy, and optionally substituted C₁₋₆-alkoxy, and R^{1*}, R^{4*}, R⁵, and R^{6*} designate hydrogen, and, more specifically, the biradical is selected from -(CH₂)₀₋₁-O-(CH₂)₁₋₃-, -(CH₂)₀₋₁-S-(CH₂)₁₋₃-, -(CH₂)₀₋₁-N(R^H)-(CH₂)₁₋₃- and -(CH₂)₁₋₄-, in particular 10 from -O-CH₂-, -S-CH₂-, -N(R^H)-CH₂-. In the latter case, the amino and thio variants appears to be particularly interesting.

In a further embodiment of the present invention, R^{2*} and R³ of an LNA incorporated into an oligomer together designate a biradical. Preferably, X is O, R² is selected from 15 hydrogen, hydroxy, and optionally substituted C₁₋₆-alkoxy, and R^{1*}, R^{4*}, R⁵, and R^{6*} designate hydrogen, and, more specifically, the biradical is selected from -(CH₂)₀₋₁-O-(CH₂)₁₋₃- and -(CH₂)₂₋₄-.

In a further embodiment of the present invention, R³ and R^{4*} of an LNA incorporated 20 into an oligomer together designate a biradical. Preferably, X is O, R^{2*} selected from hydrogen, hydroxy, and optionally substituted C₁₋₆-alkoxy, and R^{1*}, R², R⁵, and R^{6*} designate hydrogen, and, more specifically, the biradical is -(CH₂)₀₋₂-O-(CH₂)₀₋₂-.

In a further embodiment of the present invention, R³ and R^{5*} of an LNA incorporated 25 into an oligomer together designate a biradical. Preferably, X is O, R^{2*} selected from hydrogen, hydroxy, and optionally substituted C₁₋₆-alkoxy, and R^{1*}, R², R⁴, and R⁶ designate hydrogen, and, more specifically, the biradical is selected from -O-(CHR')₂₋₃- and -(CHR')₁₋₃-O-(CHR')₀₋₃-.

30 In a further embodiment of the present invention, R^{1*} and R^{4*} of an LNA incorporated into an oligomer together designate a biradical. Preferably, X is O, R^{2*} selected from hydrogen, hydroxy, and optionally substituted C₁₋₆-alkoxy, and R², R³, R⁵, and R^{6*} designate hydrogen, and, more specifically, the biradical is -(CH₂)₀₋₂-O-(CH₂)₀₋₂-.

In these embodiments, it is furthermore preferred that at least one LNA incorporated in an oligomer includes a nucleobase (substituent B) selected from adenine and guanine.

In particular, it is preferred that an oligomer have LNA incorporated therein both include at least one nucleobase selected from thymine, urasil and cytosine and at least

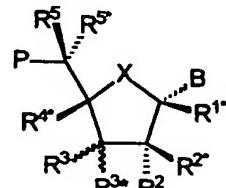
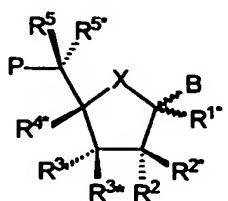
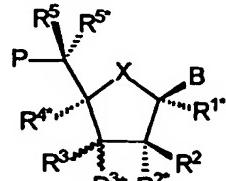
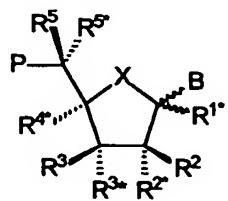
- 5 one nucleobase selected from adenine and guanine. For LNA monomers, it is especially preferred that the nucleobase is selected from adenine and guanine.

For these interesting embodiments, it is also preferred that the LNA(s) has/have the general formula Ia (see below).

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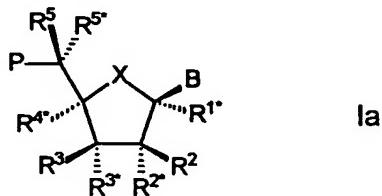
Within a variant of these interesting embodiments, all monomers of a oligonucleotide are LNA monomers.

As it will be evident from the general formula I (LNA(s) in an oligomer) (and the
15 general formula II (monomeric LNA) - see below) and the definitions associated therewith, there may be one or several asymmetric carbon atoms present in the oligomers (and monomeric LNAs) depending on the nature of the substituents and possible biradicals, cf. below. The oligomers prepared according to the method of the invention, as well as the oligomers per se, are intended to include all stereoisomers
20 arising from the presence of any and all isomers of the individual monomer fragments as well as mixtures thereof, including racemic mixtures. When considering the 5- or 6-membered ring, it is, however, believed that certain stereochemical configurations will be especially interesting, e.g. the following



where the wavy lines represent the possibility of both diastereomers arising from the interchange of the two substituents in question.

- 5 An especially interesting stereoisomeric representation is the case where the LNA(s) has/have the following formula Ia



- Also interesting as a separate aspect of the present invention is the variant of formula 10 Ia where B is in the " α -configuration".

In these cases, as well as generally, R^3 preferably designates P^+ .

- The oligomers according to the invention typically comprise 1-10000 LNA(s) of the 15 general formula I (or of the more detailed general formula Ia) and 0-10000 nucleosides selected from naturally occurring nucleosides and nucleoside analogues. The sum of the number of nucleosides and the number of LNA(s) is at least 2, preferably at least 3, in particular at least 5, especially at least 7, such as in the range of 2-15000, preferably in the range of 2-100, such as 3-100, in particular in the range of 2-50, 20 such as 3-50 or 5-50 or 7-50.

Preferably at least one LNA comprises a nucleobase as the substituent B.

- In the present context, the term "nucleoside" means a glycoside of a heterocyclic 25 base. The term "nucleoside" is used broadly as to include non-naturally occurring nucleosides, naturally occurring nucleosides as well as other nucleoside analogues. Illustrative examples of nucleosides are ribonucleosides comprising a ribose moiety as well as deoxyribonucleosides comprising a deoxyribose moiety. With respect to the bases of such nucleosides, it should be understood that this may be any of the

naturally occurring bases, e.g. adenine, guanine, cytosine, thymine, and uracil, as well as any modified variants thereof or any possible unnatural bases.

When considering the definitions and the known nucleosides (naturally occurring and 5 non-naturally occurring) and nucleoside analogues (including known bi- and tricyclic analogues), it is clear that an oligomer may comprise one or more LNA(s) (which may be identical or different both with respect to the selection of substituent and with respect to selection of biradical) and one or more nucleosides and/or nucleoside analogues. In the present context "oligonucleotide" means a successive chain of 10 nucleosides connected via internucleoside linkages, however, it should be understood that a nucleobase in one or more nucleotide units (monomers) in an oligomer (oligonucleotide) may have been modified with a substituent B as defined above.

The oligomers may be linear, branched or cyclic. In the case of a branched oligomer, 15 the branching points may be located in a nucleoside, in an internucleoside linkage or, in an intriguing embodiment, in an LNA. It is believed that in the latter case, the substituents R², R^{2*}, R³, and R^{3*} may designate two groups P* each designating an internucleoside linkage to a preceding monomer, in particular, one of R² and R^{2*} designate P* and one or R³ and R^{3*} designate a further P*. 20

As mentioned above, the LNA(s) of an oligomer are connected with other monomers via an internucleoside linkage. In the present context, the term "internucleoside linkage" means a linkage consisting of 2 to 4, preferably 3, groups/atoms selected from -CH₂- , -O-, -S-, -NR^H- , >C=O, >C=NR^H, >C=S, -Si(R'')₂- , -SO-, -S(O)₂- , -P(O)₂- , 25 -PO(BH₃)- , -P(O,S)- , -P(S)₂- , -PO(R'')- , -PO(OCH₃)- , and -PO(NHR^H)- , where R^H is selected from hydrogen and C₁₋₄-alkyl, and R'' is selected from C₁₋₆-alkyl and phenyl. Illustrative examples of such internucleoside linkages are -CH₂-CH₂-CH₂- , -CH₂-CO-CH₂- , -CH₂-CHOH-CH₂- , -O-CH₂-O- , -O-CH₂-CH₂- , -O-CH₂-CH= (including R⁵ when used as a linkage to a succeeding monomer) , -CH₂-CH₂-O- , -NR^H-CH₂-CH₂- , -CH₂-CH₂-NR^H- , -CH₂- 30 NR^H-CH₂- , -O-CH₂-CH₂-NR^H- , -NR^H-CO-O- , -NR^H-CO-NR^H- , -NR^H-CS-NR^H- , -NR^H-C(=NR^H)-NR^H- , -NR^H-CO-CH₂-NR^H- , -O-CO-O- , -O-CO-CH₂-O- , -O-CH₂-CO-O- , -CH₂-CO-NR^H- , -O-CO-NR^H- , -NR^H-CO-CH₂- , -O-CH₂-CO-NR^H- , -O-CH₂-CH₂-NR^H- , -CH=N-O- , -CH₂-NR^H-O- , -CH₂-O-N= (including R⁵ when used as a linkage to a succeeding monomer) , -CH₂-O-NR^H- , -CO-NR^H-CH₂- , -CH₂-NR^H-O- , -CH₂-NR^H-CO- , -O-NR^H-CH₂- ,

- O-NR^H-, -O-CH₂-S-, -S-CH₂-O-, -CH₂-CH₂-S-, -O-CH₂-CH₂-S-, -S-CH₂-CH= (including R⁵ when used as a linkage to a succeeding monomer), -S-CH₂-CH₂-, -S-CH₂-CH₂-O-, -S-CH₂-CH₂-S-, -CH₂-S-CH₂-, -CH₂-SO-CH₂-, -CH₂-SO₂-CH₂-, -O-SO-O-, -O-S(O)₂-O-, -O-S(O)₂-CH₂-, -O-S(O)₂-NR^H-, -NR^H-S(O)₂-CH₂-, -O-S(O)₂-CH₂-, -O-P(O)₂-O-, -O-P(O,S)-O-, -O-P(S)₂-O-, -S-P(O)₂-O-, -S-P(O,S)-O-, -S-P(S)₂-O-, -O-P(O)₂-S-, -O-P(O,S)-S-, -O-P(S)₂-S-, -S-P(O)₂-S-, -S-P(O,S)-S-, -S-P(S)₂-S-, -O-PO(R'')-O-, -O-PO(OCH₃)-O-, -O-PO(OCH₂CH₃)-O-, -O-PO(OCH₂CH₂S-R)-O-, -O-PO(BH₃)-O-, -O-PO(NHR^N)-O-, -O-P(O)₂-NR^H-, -NR^H-P(O)₂-O-, -O-P(O,NR^H)-O-, -CH₂-P(O)₂-O-, -O-P(O)₂-CH₂-, and -O-Si(R'')₂-O-; among which -CH₂-CO-NR^H-, -CH₂-NR^H-O-, -S-CH₂-O-, -O-P(O)₂-O-, -O-P(O,S)-O-, -O-P(S)₂-O-, -NR^H-P(O)₂-O-, -O-P(O,NR^H)-O-, -O-PO(R'')-O-, -O-PO(CH₃)-O-, and -O-PO(NHR^N)-O-, where R^H is selected from hydrogen and C₁₋₄-alkyl, and R'' is selected from C₁₋₆-alkyl and phenyl, are especially preferred. Further illustrative examples are given in Mesmaeker et. al., Current Opinion in Structural Biology 1995, 5, 343-355. The left-hand side of the internucleoside linkage is bound to the 5- or 6-membered ring 15 as substituent P', whereas the right-hand side is bound to the 5'-position of a preceding monomer.

- It is also clear from the above that the group P may also designate a 5'-terminal group in the case where the LNA in question is the 5'-terminal monomer. Examples of such 20 5'-terminal groups are hydrogen, hydroxy, optionally substituted C₁₋₆-alkyl, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkylcarbonyloxy, optionally substituted aryloxy, monophosphate, diphosphate, triphosphate, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C₁₋₆-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, 25 thermochemically active groups, chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B).

- In the present description and claims, the terms "monophosphate", "diphosphate", and "triphosphate" mean groups of the formula: -O-P(O)₂-O-, -O-P(O)₂-O-P(O)₂-O-, and -30 O-P(O)₂-O-P(O)₂-O-P(O)₂-O-, respectively.

In a particularly interesting embodiment, the group P designates a 5'-terminal groups selected from monophosphate, diphosphate and triphosphate. Especially the triphosphate variant is interesting as a substrate

Analogously, the group P* may designate a 3'-terminal group in the case where the LNA in question is the 3'-terminal monomer. Examples of such 3'-terminal groups are hydrogen, hydroxy, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-

- 5 alkylcarbonyloxy, optionally substituted aryloxy, and -W-A', wherein W is selected from -O-, -S-, and -N(R^H)- where R^H is selected from hydrogen and C₁₋₆-alkyl, and where A' is selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands (where the latter groups may include a spacer as defined for the substituent B).

10

In a preferred embodiment of the present invention, the oligomer has the following formula V:



15

wherein

q is 1-50;

each of n(0), ..., n(q) is independently 0-10000;

each of m(1), ..., m(q) is independently 1-10000;

- 20 with the proviso that the sum of n(0), ..., n(q) and m(1), ..., m(q) is 2-15000;

G designates a 5'-terminal group;

each Nu independently designates a nucleoside selected from naturally occurring nucleosides and nucleoside analogues;

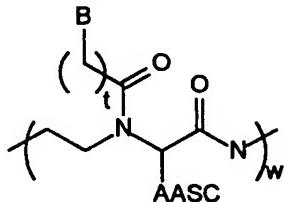
each LNA independently designates a nucleoside analogue;

- 25 each L independently designates an internucleoside linkage between two groups

selected from Nu and LNA, or L together with G* designates a 3'-terminal group; and each LNA-L independently designates a nucleoside analogue of the general formula I as defined above, or preferably of the general formula Ia as defined above.

- 30 Within this embodiment, as well as generally, the present invention provides the intriguing possibility of including LNAs with different nucleobases, in particular both nucleobases selected from thymine, cytosine and urasil and nucleobases selected from adenine and guanine.

In another embodiment of the present invention, the oligomer further comprises a PNA mono- or oligomer segment of the formula



wherein B is a defined above for the formula I, AASC designates hydrogen or an
5 amino acid side chain, t is 1-5, and w is 1-50.

In the present context, the term "amino acid side chain" means a group bound to the α -atom of an α -amino acids, i.e. corresponding to the α -amino acid in question without the glycine moiety, preferably an either naturally occurring or a readily

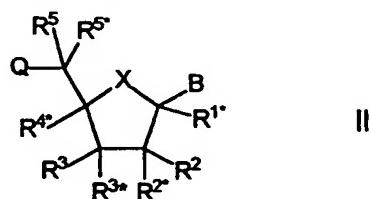
- 10 available α -amino acid. Illustrative examples are hydrogen (glycine itself), deuterium (deuterated glycine), methyl (alanine), cyanomethyl (β -cyano-alanine), ethyl, 1-propyl (norvaline), 2-propyl (valine), 2-methyl-1-propyl (leucine), 2-hydroxy-2-methyl-1-propyl (β -hydroxy-leucine), 1-butyl (norleucine), 2-butyl (isoleucine), methylthioethyl (methionine), benzyl (phenylalanine), p-amino-benzyl (p-amino-phenylalanine), p-iodo-
- 15 benzyl (p-iodo-phenylalanine), p-fluoro-benzyl (p-fluoro-phenylalanine), p-bromo-benzyl (p-bromo-phenylalanine), p-chloro-benzyl (p-chloro-phenylalanine), p-nitro-benzyl (p-nitro-phenylalanine), 3-pyridylmethyl (β -(3-pyridyl)-alanine), 3,5-diido-4-hydroxy-benzyl (3,5-diido-tyrosine), 3,5-dibromo-4-hydroxy-benzyl (3,5-dibromo-tyrosine), 3,5-dichloro-4-hydroxy-benzyl (3,5-dichloro-tyrosine), 3,5-difluoro-4-hydroxy-benzyl
- 20 (3,5-difluoro-tyrosine), 4-methoxy-benzyl (O-methyl-tyrosine), 2-naphtylmethyl (β -(2-naphtyl)-alanine), 1-naphtylmethyl (β -(1-naphtyl)-alanine), 3-indolylmethyl (tryptophan), hydroxymethyl (serine), 1-hydroxyethyl (threonine), mercaptomethyl (cysteine), 2-mercapto-2-propyl (penicillamine), 4-hydroxybenzyl (tyrosine), aminocarbonylmethyl (asparagine), 2-aminocarbonylethyl (glutamine), carboxymethyl
- 25 (aspartic acid), 2-carboxyethyl (glutamic acid), aminomethyl (α,β -diaminopropionic acid), 2-aminoethyl (α,γ -diaminobutyric acid), 3-amino-propyl (ornithine), 4-amino-1-butyl (lysine), 3-guanidino-1-propyl (arginine), and 4-imidazolylmethyl (histidine).

PNA mono- or oligomer segment may be incorporated in a oligomer as described in EP
30 0672677 A2.

The oligomers of the present invention are also intended to cover chimeric oligomers. "Chimeric oligomers" means two or more oligomers with monomers of different origin joined either directly or via a spacer. Illustrative examples of such oligomers which can 5 be combined are peptides, PNA-oligomers, oligomers containing LNA's, and oligonucleotide oligomers.

Apart from the oligomers defined above, the present invention also provides monomeric LNAs useful, e.g., in the preparation of oligomers, as substrates for, e.g., 10 nucleic acid polymerases, polynucleotide kinases, terminal transferases, and as therapeutic agents, see further below. The monomeric LNAs correspond in the overall structure (especially with respect to the possible biradicals) to the LNAs defined as constituents in oligomers, however with respect to the groups P and P⁺, the monomeric LNAs differ slightly as will be explained below. Furthermore, the 15 monomeric LNAs may comprise functional group protecting groups, especially in the cases where the monomeric LNAs are to be incorporated into oligomers by chemical synthesis.

An interesting subgroup of the possible monomeric LNAs comprises bicyclic 20 nucleoside analogues (LNAs) of the general formula II



wherein the substituent B is selected from nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; X is selected from -O-, -S-, -N(R^N)-, and -C(R⁶R⁶)-, 25 preferably from -O-, -S-, and -N(R^N)-; one of the substituents R², R²'', R³, and R³'' is a group Q*;

each of Q and Q* is independently selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, Act-O-, mercapto, Prot-S-, Act-S-, C₁₋₆-alkylthio, amino, Prot-30 N(R^H)-, Act-N(R^H)-, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy,

optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkenyloxy, optionally substituted C₂₋₆-alkynyl, optionally substituted C₂₋₆-alkynyoxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups,

- 5 reporter groups, ligands, carboxy, sulphono, hydroxymethyl, Prot-O-CH₂-, Act-O-CH₂-, aminomethyl, Prot-N(R^H)-CH₂-, Act-N(R^H)-CH₂-, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C₁₋₆-alkyl;

10

R^{2*} and R^{4*} together designate a biradical selected from -O-, -S-, -N(R^{*})-, -(CR^{*}R^{*})₊₊₊₋, -(CR^{*}R^{*}),-O-(CR^{*}R^{*})₋, -(CR^{*}R^{*}),-S-(CR^{*}R^{*})₋, -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*})₋, -O-(CR^{*}R^{*})₊₊₋O-, -S-(CR^{*}R^{*})₊₊₋O-, -O-(CR^{*}R^{*})₊₊₋S-, -N(R^{*})-(CR^{*}R^{*})₊₊₋O-, -O-(CR^{*}R^{*})₊₊₋N(R^{*})-, -S-(CR^{*}R^{*})₊₊₋S-, -N(R^{*})-(CR^{*}R^{*})₊₊₋N(R^{*})-, -N(R^{*})-(CR^{*}R^{*})₊₊₋S-, and -S-(CR^{*}R^{*})₊₊₋N(R^{*})-; R²

- 15 and R³ together designate a biradical selected from -O-, -(CR^{*}R^{*})₊₋, -(CR^{*}R^{*}),-O-(CR^{*}R^{*})₋, -(CR^{*}R^{*}),-S-(CR^{*}R^{*})₋, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*})₋; R^{2*} and R³ together designate a biradical selected from -O-, -(CR^{*}R^{*})₊₋, -(CR^{*}R^{*}),-O-(CR^{*}R^{*})₋, -(CR^{*}R^{*}),-S-(CR^{*}R^{*})₋, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*})₋; R³ and R^{4*} together designate a biradical selected from -(CR^{*}R^{*}),-O-(CR^{*}R^{*})₋, -(CR^{*}R^{*}),-S-(CR^{*}R^{*})₋, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*})₋;

- 20 R³ and R⁵ together designate a biradical selected from -(CR^{*}R^{*}),-O-(CR^{*}R^{*})₋, -(CR^{*}R^{*}),-S-(CR^{*}R^{*})₋, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*})₋; R^{1*} and R^{4*} together designate a biradical selected from -(CR^{*}R^{*}),-O-(CR^{*}R^{*})₋, -(CR^{*}R^{*}),-S-(CR^{*}R^{*})₋, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*})₋; or R^{1*} and R^{2*} together designate a biradical selected from -(CR^{*}R^{*}),-O-(CR^{*}R^{*})₋,

- 25 -(CR^{*}R^{*}),-S-(CR^{*}R^{*})₋, and -(CR^{*}R^{*}),-N(R^{*})-(CR^{*}R^{*})₋; wherein R^{*} is as defined above for the oligomers; and each of the substituents R^{1*}, R², R^{2*}, R³, R^{4*}, R⁵, and R^{5*}, which are not involved in O, Q^{*} or the biradical, are as defined above for the oligomers.

- It should furthermore be understood, with due consideration of the known bicyclic nucleoside analogues, that R² and R³ do not together designate a biradical selected from -O-CH₂-CH₂- and -O-CH₂-CH₂-CH₂-; and R³ and R⁵ do not together designate a biradical selected from -CH₂-CH₂-, -O-CH₂-, and -O-Si(Pr)₂-O-Si(Pr)₂-O-.

The monomeric LNAs also comprise basic salts and acid addition salts thereof.

Furthermore, it should be understood that any chemical group (including any

nucleobase), which is reactive under the conditions prevailing in chemical oligonucleotide synthesis, is optionally functional group protected as known in the art.

This means that groups such as hydroxy, amino, carboxy, sulphono, and mercapto groups, as well as nucleobases, of a monomeric LNA are optionally functional group

- 5 protected. Protection (and deprotection) is performed by methods known to the person skilled in the art (see, e.g., Greene, T. W. and Wuts, P. G. M., "Protective Groups in Organic Synthesis", 2nd ed., John Wiley, N.Y. (1991), and M.J. Gait, *Oligonucleotide Synthesis*, IRL Press, 1984).

- 10 Illustrative examples of hydroxy protection groups are optionally substituted trityl, such as 4,4'-dimethoxytrityl (DMT), 4-monomethoxytrityl (MMT), and trityl, optionally substituted 9-(9-phenyl)xanthenyl (pixyl), optionally substituted ethoxycarbonyloxy, *p*-phenylazophenoxy carbonyloxy, tetrahydropyranyl (thp), 9-fluorenylmethoxycarbonyl (Fmoc), methoxytetrahydropyranyl (mthp), silyloxy such as trimethylsilyl (TMS),
- 15 triisopropylsilyl (TIPS), *tert*-butyldimethylsilyl (TBDMS), triethylsilyl, and phenyldimethylsilyl, benzyloxycarbonyl or substituted benzyloxycarbonyl ethers such as 2-bromo benzyloxycarbonyl, *tert*-butylethers, alkyl ethers such as methyl ether, acetals (including two hydroxy groups), acyloxy such as acetyl or halogen substituted acetyls, e.g. chloroacetyl or fluoroacetyl, isobutyryl, pivaloyl, benzoyl and substituted
- 20 benzoyls, methoxymethyl (MOM), benzyl ethers or substituted benzyl ethers such as 2,6-dichlorobenzyl (2,6-Cl₂Bzl). Alternatively, the hydroxy group may be protected by attachment to a solid support optionally through a linker.

Illustrative examples of amino protection groups are Fmoc (fluorenylmethoxycarbonyl),

- 25 BOC (*tert*-butyloxycarbonyl), trifluoroacetyl, allyloxycarbonyl (alloc, AOC), benzyl oxycarbonyl (Z, Cbz), substituted benzyloxycarbonyls such as 2-chlorobenzyloxycarbonyl ((2-ClZ), monomethoxytrityl (MMT), dimethoxytrityl (DMT), phthaloyl, and 9-(9-phenyl)xanthenyl (pixyl)).

- 30 Illustrative examples of carboxy protection groups are allyl esters, methyl esters, ethyl esters, 2-cyanoethylesters, trimethylsilylethylesters, benzyl esters (Obzl), 2-adamantyl esters (O-2-Ada), cyclohexyl esters (OcHex), 1,3-oxazolines, oxazoles, 1,3-oxazolidines, amides or hydrazides.

Illustrative examples of mercapto protecting groups are trityl (Trt), acetamidomethyl (acm), trimethylacetamidomethyl (Tacm), 2,4,6-trimethoxybenzyl (Tmob), *tert*-butylsulfenyl (StBu), 9-fluorenylmethyl (Fm), 3-nitro-2-pyridinesulfenyl (Npys), and 4-methylbenzyl (Meb).

5

Furthermore, it may be necessary or desirable to protect any nucleobase included in an monomeric LNA, especially when the monomeric LNA is to be incorporated in an oligomer according to the invention. In the present context, the term "protected nucleobases" means that the nucleobase in question is carrying a protection group

- 10 selected among the groups which are well-known for a man skilled in the art (see e.g. Protocols for Oligonucleotides and Analogs, vol 20, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, 49, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223; and E. Uhlmann and A. Peyman, *Chem. Rev.*, 90, 543.). Illustrative examples are benzoyl, isobutyryl,
- 15 *tert*-butyl, *tert*-butyloxycarbonyl, 4-chloro-benzyloxycarbonyl, 9-fluorenylmethyl, 9-fluorenylmethyloxycarbonyl, 4-methoxybenzoyl, 4-methoxytriphenylmethyl, optionally substituted triazolo, *p*-toluenesulphonyl, optionally substituted sulphonyl, isopropyl, optionally substituted amidines, optionally substituted trityl, phenoxyacetyl, optionally substituted acyl, pixyl, tetrahydropyranyl, optionally substituted silyl ethers, and 4-
- 20 methoxybenzyloxycarbonyl. Chapter 1 in "Protocols for oligonucleotide conjugates", Methods in Molecular Biology, vol 26, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ. and S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223 disclose further suitable examples.
- 25 In a preferred embodiment, the group B in a monomeric LNA is preferably selected from nucleobases and protected nucleobases.

- In an embodiment of the monomeric LNAs according to the present invention, one of Q and Q^{*}, preferably Q^{*}, designates a group selected from Act-O-, Act-S-, Act-N(R^H)-,
- 30 Act-O-CH₂-, Act-S-CH₂-, Act-N(R^H)-CH₂-, and the other of Q and Q^{*}, preferably Q, designates a group selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, mercapto, Prot-S-, C₁₋₆-alkylthio, amino, Prot-N(R^H)-, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkenyloxy, optionally

substituted C₂₋₆-alkynyl, optionally substituted C₂₋₆-alkynyloxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphonato, hydroxymethyl, Prot-O-CH₂-, aminomethyl, Prot-N(R^H)-CH₂-, carboxymethyl,

- 5 sulphonomethyl, and R^H is selected from hydrogen and C₁₋₆-alkyl.

In the case described above, the group Prot designates a protecting group for -OH, -SH, and -NH(R^H), respectively. Such protection groups are selected from the same as defined above for hydroxy protection groups, mercapto protection group, and amino

- 10 protection groups, respectively, however taking into consideration the need for a stable and reversible protection group. However, it is preferred that any protection group for -OH is selected from optionally substituted trityl, such as dimethoxytrityl (DMT), monomethoxytrityl (MMT), and trityl, and 9-(9-phenyl)xanthenyl (pixyl),

optionally substituted, tetrahydropyranyl (thp) (further suitable hydroxy protection

- 15 groups for phosphoramidite oligonucleotide synthesis are described in Agrawal, ed.

"Protocols for Oligonucleotide Conjugates"; Methods in Molecular Biology, vol. 26, Humana Press, Totowa, NJ (1994) and Protocols for Oligonucleotides and Analogs, vol 20, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ), or protected as acetal; that any protection group for -SH is selected from trityl, such as

- 20 dimethoxytrityl (DMT), monomethoxytrityl (MMT), and trityl, and 9-(9-phenyl)xanthenyl (pixyl), optionally substituted, tetrahydropyranyl (thp) (further

suitable mercapto protection groups for phosphoramidite oligonucleotide synthesis are also described in Agrawal (see above); and that any protecting group for -NH(R^H) is selected from trityl, such as dimethoxytrityl (DMT), monomethoxytrityl (MMT), and

- 25 trityl, and 9-(9-phenyl)xanthenyl (pixyl), optionally substituted, tetrahydropyranyl (thp) (further suitable amino protection groups for phosphoramidite oligonucleotide synthesis are also described in Agrawal (see above).

In the embodiment above, as well as for any monomeric LNAs defined herein, Act

- 30 designates an activation group for -OH, -SH, and -NH(R^H), respectively. Such activation groups are, e.g., selected from optionally substituted O-phosphoramidite, optionally substituted O-phosphotriester, optionally substituted O-phosphodiester, optionally substituted H-phosphonate, and optionally substituted O-phosphonate.

- In the present context, the term "phosphoramidite" means a group of the formula -P(OR^x)-N(R^y)₂, wherein R^x designates an optionally substituted alkyl group, e.g. methyl, 2-cyanoethyl, or benzyl, and each of R^y designate optionally substituted alkyl groups, e.g. ethyl or isopropyl, or the group -N(R^y)₂ forms a morpholino group
- 5 (-N(CH₂CH₂)₂O). R^x preferably designates 2-cyanoethyl and the two R^y are preferably identical and designate isopropyl. Thus, an especially relevant phosphoramidite is N,N-diisopropyl-O-(2-cyanoethyl)phosphoramidite.

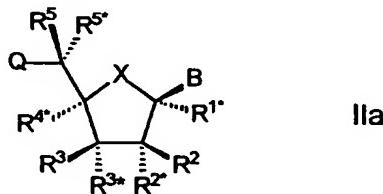
It should be understood that the protecting groups used herein for a single monomeric
10 LNA or several monomeric LNAs may be selected so that when this/these LNA(s) are incorporated in an oligomer according to the invention, it will be possible to perform either a simultaneous deprotection or a sequential deprotection of the functional groups. The latter situation opens for the possibility of regioselectively introducing one or several "active/functional" groups such as DNA intercalators, photochemically
15 active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, where such groups may be attached via a spacer as described above.

In a preferred embodiment, Q is selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Prot-O-, mercapto, Prot-S-, C₁₋₆-alkylthio, amino, Prot-N(R^H)-, mono- or di(C₁₋₆-20 alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkenyloxy, optionally substituted C₂₋₆-alkynyl, optionally substituted C₂₋₆-alkynyoxy, monophosphate, diphosphate, triphosphate, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy,
25 sulpho, hydroxymethyl, Prot-O-CH₂-, aminomethyl, Prot-N(R^H)-CH₂-, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C₁₋₆-alkyl; and Q^{*} is selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, Act-O-, mercapto, Act-S-, C₁₋₆-alkylthio, amino, Act-N(R^H)-, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-30 alkoxy, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkenyloxy, optionally substituted C₂₋₆-alkynyl, optionally substituted C₂₋₆-alkynyoxy, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulpho, where

Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H is selected from hydrogen and C₁₋₆-alkyl.

The monomeric LNAs of the general formula II may, as the LNAs incorporated into 5 oligomers, represent various stereoisomers. Thus, the stereochemical variants described above for the LNAs incorporated into oligomers are believed to be equally applicable in the case of monomeric LNAs (however, it should be noted that P should then be replaced with Q).

- 10 In a preferred embodiment of the present invention, the monomeric LNA has the general formula IIa



wherein the substituents are defined as above.

15

Furthermore, with respect to the definitions of substituents, biradicals, R[•], etc. the same preferred embodiments as defined above for the oligomer according to the invention also apply in the case of monomeric LNAs.

- 20 In a particularly interesting embodiment of the monomeric LNAs of the present invention, B designates a nucleobase, preferably a nucleobase selected from thymine, cytosine, urasil, adenine and guanine (in particular adenine and guanine), X is -O-, R^{2*} and R^{4*} together designate a biradical selected from -(CH₂)₀₋₁-O-(CH₂)₁₋₃-, -(CH₂)₀₋₁-S-(CH₂)₁₋₃-, and -(CH₂)₀₋₁-N(R^N)-(CH₂)₁₋₃-, in particular -O-CH₂-, -S-CH₂- and -R^N-CH₂-, 25 where R^N is selected from hydrogen and C₁₋₄-alkyl, Q designates Prot-O-, R^{3*} is Q[•] which designates Act-OH, and R^{1*}, R², R³, R⁵, and R^{5*} each designate hydrogen. In this embodiment, R^N may also be selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups and ligands.

- In a further particularly interesting embodiment of the monomeric LNAs of the present invention, B designates a nucleobase, preferably a nucleobase selected from thymine, cytosine, urasil, adenine and guanine (in particular adenine and guanine), X is -O-, R²⁺ and R⁴⁺ together designate a biradical selected from -(CH₂)₀₋₁-O-(CH₂)₁₋₃-, -(CH₂)₀₋₁-
- 5 S-(CH₂)₁₋₃-, and -(CH₂)₀₋₁-N(R^N)-(CH₂)₁₋₃-, in particular -O-CH₂-, -S-CH₂- and -R^N-CH₂-, where R^N is selected from hydrogen and C₁₋₄-alkyl, Q is selected from hydroxy, mercapto, C₁₋₆-alkylthio, amino, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₂₋₆-alkenyloxy, optionally substituted C₂₋₆-alkynyloxy, monophosphate, diphosphate, and triphosphate, R³⁺ is Q⁺ which is
- 10 selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, mercapto, C₁₋₆-alkylthio, amino, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkenyloxy, optionally substituted C₂₋₆-alkynyl, and optionally substituted C₂₋₆-alkynyloxy, R³ is selected from hydrogen, optionally substituted C₁₋₆-
- 15 alkyl, optionally substituted C₂₋₆-alkenyl, and optionally substituted C₂₋₆-alkynyl, and R¹⁺, R², R⁵, and R⁶⁺ each designate hydrogen. Also here, R^N may also be selected from DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups and ligands.
- 20 In a further particularly interesting embodiment of the monomeric LNAs of the present invention, B designates a nucleobase, X is -O-, R² and R³ together designate a biradical selected from -(CH₂)₀₋₁-O-CH=CH-, -(CH₂)₀₋₁-S-CH=CH-, and -(CH₂)₀₋₁-N(R^N)-CH=CH- where R^N is selected from hydrogen and C₁₋₄-alkyl, Q is selected from hydroxy, mercapto, C₁₋₆-alkylthio, amino, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy,
- 25 C₁₋₆-alkenyloxy, monophosphate, diphosphate, and triphosphate, R³⁺ is Q⁺ which is selected from hydrogen, azido, halogen, cyano, nitro, hydroxy, mercapto, C₁₋₆-alkylthio, amino, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted C₂₋₆-alkenyloxy, optionally substituted C₂₋₆-alkynyl, and optionally substituted C₂₋₆-alkynyloxy, and R¹⁺, R²+, R⁴+, R⁵, and R⁶⁺ each designate hydrogen.
- 30

One aspect of the invention is to provide various derivatives of LNAs for solid-phase and/or solution phase incorporation into an oligomer. As an illustrative example,

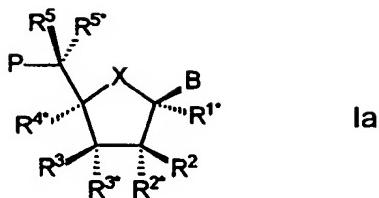
monomers suitable for incorporation of (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(cytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(urasil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane,

5 (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(guanin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, and (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(adenin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane using the phosphoramidite approach, the phosphortriester approach, and the *H*-phosphonate approach, respectively, are (*1R,3R,4R,7S*)-7-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4'-dimethoxytrityloxymethyl)-3-

10 (*thymin-1-yl*)-2,5-dioxabicyclo[2.2.1]heptane, (*1R,3R,4R,7S*)-7-hydroxy-1-(4,4'-dimethoxytrityloxymethyl)-3-(*thymin-1-yl*)-2,5-dioxabicyclo[2.2.1]heptane-7-*O*-(2-chlorophenylphosphate), and (*1R,3R,4R,7S*)-7-hydroxy-1-(4,4'-dimethoxytrityloxymethyl)-3-(*thymin-1-yl*)-2,5-dioxabicyclo[2.2.1]heptane-7-*O*-(*H*-phosphonate) and the 3-(cytosin-1-yl), 3-(urasil-1-yl), 3-(adenin-1-yl) and 3-(guanin-1-yl) analogues thereof, respectively. Furthermore, the analogues where the methyleneoxy biradical of the monomers is substituted with a methylenethio, a methyleneamino, or a 1,2-ethylene biradical are also expected to constitute particularly interesting variants within the present invention. The methylenethio and methyleneamino analogues are believed to equally applicable as the methyleneoxy analogue and therefore the specific reagents corresponding to those mentioned for incorporation of (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(cytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(urasil-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(guanin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane, and (*1S,3R,4R,7S*)-7-hydroxy-1-hydroxymethyl-3-(adenin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane should also be considered as particularly interesting reactive monomers within the present invention. For the methyleneamine analogue, it should be noted that the secondary amine may carry a substituent selected from optionally substituted C₁₋₆-alkyl such as methyl and benzyl,

20 optionally substituted C₁₋₆-alkylcarbonyl such as trifluoroacetyl, optionally substituted arylcarbonyl and optionally substituted heteroarylcarbonyl.

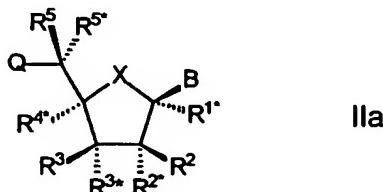
25 In a particularly interesting embodiment, the present invention relates to an oligomer comprising at least one LNA of the general formula Ia



wherein X is -O-; B is selected from nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R⁵; R^{3*} is a group P* which designates an internucleoside linkage to a preceding monomer, or a 3'-terminal group; R^{2*} and R^{4*} together designate a biradical selected from -O-, -S-, -N(R^{*})-, -(CR^{*}R^{*})_{r+s+1}-, -(CR^{*}R^{*})_r-O-(CR^{*}R^{*})_s-, -(CR^{*}R^{*})_s-N(R^{*})-(CR^{*}R^{*})_r-, -O-(CR^{*}R^{*})_{r+s}-O-, -S-(CR^{*}R^{*})_{r+s}-O-, -O-(CR^{*}R^{*})_{r+s}-S-, -N(R^{*})-(CR^{*}R^{*})_{r+s}-N(R^{*})-, -N(R^{*})-(CR^{*}R^{*})_{r+s}-S-, and -S-(CR^{*}R^{*})_{r+s}-N(R^{*})-; wherein each R^{*} is independently selected from hydrogen, halogen, azido, cyano, nitro, hydroxy, mercapto, amino, mono- or di(C₁₋₆-alkyl)amino, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and/or two adjacent (non-geminal) R^{*} may together designate a double bond, and each of r and s is 0-3 with the proviso that the sum r+s is 1-4; each of the substituents R^{1*}, R², R³, R⁵, and R^{3*} is independently selected from hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, hydroxy, C₁₋₆-alkoxy, C₂₋₆-alkenyloxy, carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, C₁₋₆-alkyl-carbonylamino, carbamido, azido, C₁₋₆-alkanoyloxy, sulphono, sulphanyl, C₁₋₆-alkylthio, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and halogen, where two geminal substituents together may designate oxo; and basic salts and acid addition salts thereof. In particular, one R^{*} is selected from hydrogen, hydroxy, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and any remaining substituents R^{*} are hydrogen.

Especially, the biradical is selected from $-O\cdot$, $-(CH_2)_{0-1}O-(CH_2)_{1-3}\cdot$, $-(CH_2)_{0-1}S-(CH_2)_{1-3}\cdot$, $-(CH_2)_{0-1}N(R^N)-(CH_2)_{1-3}\cdot$, and $-(CH_2)_{2-4}\cdot$.

In a further particularly interesting embodiment, the present invention relates to an
5 LNA of the general formula IIa



- wherein X is $-O\cdot$; B is selected from nucleobases, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands; $R^3\cdot$ is a group Q^* ; each of Q and Q^* is independently selected from hydrogen,
10 azido, halogen, cyano, nitro, hydroxy, Prot-O-, Act-O-, mercapto, Prot-S-, Act-S-, C_{1-6} -alkylthio, amino, Prot-N(R^H)-, Act-N(R^H)-, mono- or di(C_{1-6} -alkyl)amino, optionally substituted C_{1-6} -alkoxy, optionally substituted C_{1-6} -alkyl, optionally substituted C_{2-6} -alkenyl, optionally substituted C_{2-6} -alkenoxy, optionally substituted C_{2-6} -alkynyl, optionally substituted C_{2-6} -alkynyloxy, monophosphate, diphosphate, triphosphate,
15 DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, ligands, carboxy, sulphonato, hydroxymethyl, Prot-O- $CH_2\cdot$, Act-O- $CH_2\cdot$, aminomethyl, Prot-N(R^H)- $CH_2\cdot$, Act-N(R^H)- $CH_2\cdot$, carboxymethyl, sulphonomethyl, where Prot is a protection group for -OH, -SH, and -NH(R^H), respectively, Act is an activation group for -OH, -SH, and -NH(R^H), respectively, and R^H
20 is selected from hydrogen and C_{1-6} -alkyl; $R^2\cdot$ and $R^4\cdot$ together designate a biradical selected from $-O\cdot$, $-S\cdot$, $-N(R^{\cdot})\cdot$, $-(CR^{\cdot}R^{\cdot})_{r+s+1}\cdot$, $-(CR^{\cdot}R^{\cdot})_rO-(CR^{\cdot}R^{\cdot})_s\cdot$, $-(CR^{\cdot}R^{\cdot})_rS-(CR^{\cdot}R^{\cdot})_s\cdot$, $-(CR^{\cdot}R^{\cdot})_rN(R^{\cdot})-(CR^{\cdot}R^{\cdot})_s\cdot$, $-O-(CR^{\cdot}R^{\cdot})_{r+s}-O\cdot$, $-S-(CR^{\cdot}R^{\cdot})_{r+s}-O\cdot$, $-O-(CR^{\cdot}R^{\cdot})_{r+s}S-$, $-N(R^{\cdot})-(CR^{\cdot}R^{\cdot})_{r+s}-O\cdot$, $-O-(CR^{\cdot}R^{\cdot})_{r+s}N(R^{\cdot})-$, $-S-(CR^{\cdot}R^{\cdot})_{r+s}S-$, $-N(R^{\cdot})-(CR^{\cdot}R^{\cdot})_{r+s}-N(R^{\cdot})-$, $-N(R^{\cdot})-(CR^{\cdot}R^{\cdot})_{r+s}-S-$, and $-S-(CR^{\cdot}R^{\cdot})_{r+s}-N(R^{\cdot})\cdot$; wherein each R^{\cdot} is independently selected from
25 hydrogen, halogen, azido, cyano, nitro, hydroxy, mercapto, amino, mono- or di(C_{1-6} -alkyl)amino, optionally substituted C_{1-6} -alkoxy, optionally substituted C_{1-6} -alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and/or two adjacent (non-geminal) R^{\cdot} may together designate a double bond, and each of r and s is 0-3 with the proviso
30 that the sum $r+s$ is 1-4; each of the substituents $R^1\cdot$, $R^2\cdot$, $R^3\cdot$, $R^5\cdot$, and $R^5\cdot$ is

- independently selected from hydrogen, optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, hydroxy, C₁₋₆-alkoxy, C₂₋₆-alkenyloxy, carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)-amino-carbonyl, C₁₋₆-alkyl-carbonylamino,
- 5 carbamido, azido, C₁₋₆-alkanoyloxy, sulphono, sulphanyl, C₁₋₆-alkylthio, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and halogen, where two geminal substituents together may designate oxo; and basic salts and acid addition salts thereof; and with the proviso that any chemical group (including any nucleobase),
- 10 which is reactive under the conditions prevailing in oligonucleotide synthesis, is optionally functional group protected. Preferably, one R' is selected from hydrogen, hydroxy, optionally substituted C₁₋₆-alkoxy, optionally substituted C₁₋₆-alkyl, DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands, and any remaining substituents R' are
- 15 hydrogen. Especially, the biradical is selected from -O-, -(CH₂)₀₋₁-O-(CH₂)₁₋₃-, -(CH₂)₀₋₁-S-(CH₂)₁₋₃-, -(CH₂)₀₋₁-N(R^N)-(CH₂)₁₋₃-, and -(CH₂)₂₋₄-.

Generally, the present invention provides oligomers having surprisingly good hybridisation properties with respect to affinity and specificity. Thus, the present

20 invention provides an oligomer comprising at least one nucleoside analogue which imparts to the oligomer a T_m with a complementary DNA oligonucleotide which is at least 2.5 °C higher, preferably at least 3.5 °C higher, in particular at least 4.0 °C higher, especially at least 5.0 °C higher, than that of the corresponding unmodified reference oligonucleotide which does not comprise any nucleoside analogue. In

25 particular, the T_m of the oligomer is at least 2.5 x N °C higher, preferably at least 3.5 x N °C higher, in particular at least 4.0 x N °C higher, especially at least 5.0 x N°C higher, where N is the number of nucleoside analogues.

In the case of hybridisation with a complementary RNA oligonucleotide, the at least

30 one nucleoside analogue imparts to the oligomer a T_m with the complementary DNA oligonucleotide which is at least 4.0 °C higher, preferably at least 5.0 °C higher, in particular at least 6.0 °C higher, especially at least 7.0 °C higher, than that of the corresponding unmodified reference oligonucleotide which does not comprise any nucleoside analogue. In particular, the T_m of the oligomer is at least 4.0 x N °C higher,

preferably at least $5.0 \times N^{\circ}C$ higher, in particular at least $6.0 \times N^{\circ}C$ higher, especially at least $7.0 \times N^{\circ}C$ higher, where N is the number of nucleoside analogues.

The term "corresponding unmodified reference oligonucleotide" is intended to mean an
5 oligonucleotide solely consisting of naturally occurring nucleotides which represents
the same nucleobases in the same absolute order (and the same orientation).

The T_m is measured under one of the following conditions (i.e. essentially as illustrated
in Example 129):

10

- a) 10mM Na₂HPO₄, pH 7.0, 100mM NaCl, 0.1mM EDTA;
- b) 10mM Na₂HPO₄ pH 7.0, 0.1mM EDTA; or
- c) 3M tetramethylammoniumchlorid (TMAC), 10mM Na₂HPO₄, pH 7.0, 0.1mM EDTA;

15 preferably under conditions a), at equimolar amounts (typically 1.0 μ M) of the
oligomer and the complementary DNA oligonucleotide.

The oligomer is preferably as defined above, where the at least one nucleoside
analogue has the formula I where B is a nucleobase. In particular interesting is the
20 cases where at least one nucleoside analogue includes a nucleobase selected from
adenine and guanine.

Furthermore, with respect to specificity and affinity, the oligomer, when hybridised
with a partially complementary DNA oligonucleotide, or a partially complementary RNA
25 oligonucleotide, having one or more mismatches with said oligomer, should exhibit a
reduction in T_m , as a result of said mismatches, which is equal to or greater than the
reduction which would be observed with the corresponding unmodified reference
oligonucleotide which does not comprise any nucleoside analogues. Also, the oligomer
should have substantially the same sensitivity of T_m to the ionic strength of the
30 hybridisation buffer as that of the corresponding unmodified reference oligonucleotide.

Oligomers defined herein are typically at least 30% modified, preferably at least 50%
modified, in particular 70% modified, and in some interesting applications 100%
modified.

The oligomers of the invention has substantially higher 3'-exonucleolytic stability than the corresponding unmodified reference oligonucleotide. This important property can be examined as described in Example 136.

5

Definitions

In the present context, the term "C₁₋₁₂-alkyl" means a linear, cyclic or branched hydrocarbon group having 1 to 12 carbon atoms, such as methyl, ethyl, propyl, *iso*-

10 propyl, cyclopropyl, butyl, *tert*-butyl, *iso*-butyl, cyclobutyl, pentyl, cyclopentyl, hexyl, cyclohexyl, and dodecyl. Analogously, the term "C₁₋₆-alkyl" means a linear, cyclic or branched hydrocarbon group having 1 to 6 carbon atoms, such as methyl, ethyl, propyl, *iso*-propyl, pentyl, cyclopentyl, hexyl, cyclohexyl, and the term "C₁₋₄-alkyl" is intended to cover linear, cyclic or branched hydrocarbon groups having 1 to 4 carbon
15 atoms, e.g. methyl, ethyl, propyl, *iso*-propyl, cyclopropyl, butyl, *iso*-butyl, *tert*-butyl, cyclobutyl.

Preferred examples of "C₁₋₆-alkyl" are methyl, ethyl, propyl, *iso*-propyl, butyl, *tert*-butyl, *iso*-butyl, pentyl, cyclopentyl, hexyl, cyclohexyl, in particular methyl, ethyl,

20 propyl, *iso*-propyl, *tert*-butyl, *iso*-butyl and cyclohexyl. Preferred examples of "C₁₋₄-alkyl" are methyl, ethyl, propyl, *iso*-propyl, butyl, *tert*-butyl, and *iso*-butyl.

Similarly, the term "C₂₋₁₂-alkenyl" covers linear, cyclic or branched hydrocarbon groups having 2 to 12 carbon atoms and comprising one unsaturated bond. Examples of

25 alkenyl groups are vinyl, allyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, decaenyl. Analogously, the term "C₂₋₆-alkenyl" is intended to cover linear, cyclic or branched hydrocarbon groups having 2 to 6 carbon atoms and comprising one unsaturated bond. Preferred examples of alkenyl are vinyl, allyl, butenyl, especially allyl.

30

Similarly, the term "C₂₋₁₂-alkynyl" means a linear or branched hydrocarbon group having 2 to 12 carbon atoms and comprising a triple bond. Examples hereof are ethynyl, propynyl, butynyl, octynyl, and dodecanyl.

- In the present context, *i.e.* in connection with the terms "alkyl", "alkenyl", and "alkynyl", the term "optionally substituted" means that the group in question may be substituted one or several times, preferably 1-3 times, with group(s) selected from hydroxy (which when bound to an unsaturated carbon atom may be present in the
- 5 tautomeric keto form), C₁₋₆-alkoxy (*i.e.* C₁₋₆-alkyl-oxy), C₂₋₆-alkenyloxy, carboxy, oxo (forming a keto or aldehyde functionality), C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, aryloxy, arylcarbonyl, heteroaryl, heteroaryloxycarbonyl, heteroaryloxy, heteroarylcarbonyl, amino, mono- and di(C₁₋₆-alkyl)amino; carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkylcarbonylamino, cyano, guanidino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, sulphanyl, C₁₋₆-alkylthio, halogen, where any aryl and heteroaryl may be substituted as specifically describe below for "optionally substituted aryl and heteroaryl".
- 15 Preferably, the substituents are selected from hydroxy, C₁₋₆-alkoxy, carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C₁₋₆-alkyl)amino, carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkylcarbonylamino, cyano, carbamido, halogen, where aryl and heteroaryl may be substituted 1-5 times, preferably 1-3 times, with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino or halogen. Especially preferred examples are hydroxy, C₁₋₆-alkoxy, carboxy, aryl, heteroaryl, amino, mono- and di(C₁₋₆-alkyl)amino, and halogen, where aryl and heteroaryl may be substituted 1-3 times with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino or halogen.
- 25 In the present context the term "aryl" means a fully or partially aromatic carbocyclic ring or ring system, such as phenyl, naphthyl, 1,2,3,4-tetrahydronaphthyl, anthracyl, phenanthracyl, pyrenyl, benzopyrenyl, fluorenyl and xanthenyl, among which phenyl is a preferred example.
- 30 The term "heteroaryl" means a fully or partially aromatic carbocyclic ring or ring system where one or more of the carbon atoms have been replaced with heteroatoms, *e.g.* nitrogen (=N- or -NH), sulphur, and/or oxygen atoms. Examples of such heteroaryl groups are oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrrolyl, imidazolyl,

pyrazolyl, pyridinyl, pyrazinyl, pyridazinyl, piperidinyl, coumaryl, furyl, quinolyl, benzothiazolyl, benzotriazolyl, benzodiazolyl, benzooxozolyl, phthalazinyl, phthalanyl, triazolyl, tetrazolyl, isoquinolyl, acridinyl, carbazolyl, dibenzazepinyl, indolyl, benzopyrazolyl, phenoxazonyl.

5

In the present context, *i.e.* in connection with the terms "aryl" and "heteroaryl", the term "optionally substituted" means that the group in question may be substituted one or several times, preferably 1-5 times, in particular 1-3 times) with group(s) selected from hydroxy (which when present in an enol system may be represented in the

- 10 tautomeric keto form), C₁₋₆-alkyl, C₁₋₆-alkoxy, oxo (which may be represented in the tautomeric enol form), carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, formyl, aryl, aryloxy, aryloxycarbonyl, arylcarbonyl, heteroaryl, amino, mono- and di(C₁₋₆-alkyl)amino; carbamoyl, mono- and di(C₁₋₆-alkyl)aminocarbonyl, amino-C₁₋₆-alkyl-aminocarbonyl, mono- and di(C₁₋₆-alkyl)amino-C₁₋₆-alkyl-aminocarbonyl, C₁₋₆-alkylcarbonylamino, cyano, guanidino, carbamido, C₁₋₆-alkanoyloxy, sulphono, C₁₋₆-alkylsulphonyloxy, nitro, sulphanyl, dihalogen-C₁₋₄-alkyl, trihalogen-C₁₋₄-alkyl, halogen, where aryl and heteroaryl representing substituents may be substituted 1-3 times with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino or halogen. Preferred examples are hydroxy, C₁₋₆-alkyl, C₁₋₆-alkoxy, carboxy, C₁₋₆-alkoxycarbonyl, C₁₋₆-alkylcarbonyl, aryl, amino,
- 15 20 mono- and di(C₁₋₆-alkyl)amino, and halogen, wherein aryl may be substituted 1-3 times with C₁₋₄-alkyl, C₁₋₄-alkoxy, nitro, cyano, amino or halogen.

"Halogen" includes fluoro, chloro, bromo, and iodo.

- 25 It should be understood that oligomers (wherein LNAs are incorporated) and LNAs as such include possible salts thereof, of which pharmaceutically acceptable salts are especially relevant. Salts include acid addition salts and basic salts. Examples of acid addition salts are hydrochloride salts, sodium salts, calcium salts, potassium salts, etc.. Examples of basic salts are salts where the (remaining) counter ion is selected
- 30 from alkali metals, such as sodium and potassium, alkaline earth metals, such as calcium, and ammonium ions (+N(R^a)₃R^b, where each of R^a and R^b independently designates optionally substituted C₁₋₆-alkyl, optionally substituted C₂₋₆-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl). Pharmaceutically acceptable salts are, *e.g.*, those described in Remington's Pharmaceutical Sciences,

17. Ed. Alfonso R. Gennaro (Ed.), Mack Publishing Company, Easton, PA, U.S.A.,
1985 and more recent editions and in Encyclopedia of Pharmaceutical Technology.
Thus, the term "an acid addition salt or a basic salt thereof" used herein is intended to
comprise such salts. Furthermore, the oligomers and LNAs as well as any
5 intermediates or starting materials therefor may also be present in hydrate form.

Preparation of monomers

In a preferred embodiment, nucleosides containing an additional 2'-O,4'-C-linked ring
10 were synthesised by the following procedure:

Synthesis of a number of 4'-C-hydroxymethyl nucleosides have been reported earlier
(R. D. Youssefeyeh, J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, 1979, 44,
1301; G. H. Jones, M. Taniguchi, D. Tegg and J. G. Moffatt, *J. Org. Chem.*, 1979,
15 44, 1309; C. O-Yang, H. Y. Wu, E. B. Fraser-Smith and K. A. M. Walker, *Tetrahedron Lett.*, 1992, 33, 37; H. Thrane, J. Fensholdt, M. Regner and J. Wengel, *Tetrahedron*,
1995, 51, 10389; K. D. Nielsen, F. Kirpekar, P. Roepstorff and J. Wengel, *Bioorg. Med. Chem.*, 1995, 3, 1493). For exemplification of synthesis of 2'-O,4'-C-linked
bicyclic nucleosides we chose a strategy starting from 4'-C-hydroxymethyl furanose
20 derivative 31. Benzylation, acetylation, and acetolysis followed by another acetylation
afforded furanose 33, a key intermediate for nucleoside coupling. Stereoselective
reaction with silylated thymine afforded compound 34 which was deacetylated to give
nucleoside diol 35. Tosylation followed by base-induced ring closure afforded the 2'-
O,4'-C-linked bicyclic nucleoside derivative 36. Debenzylation yielded the unprotected
25 bicyclic nucleoside analogue 37 which was transformed into the 5'-O-4,4'-
dimethoxytrityl protected analogue 38 and subsequently into the phosphoramidite
derivative 39 for oligonucleotide synthesis. A similar procedure has been used for
synthesis of the corresponding uracil, adenine, cytosine and guanine nucleosides as
exemplified in the example section. This coupling method is only one of several
30 possible as will be apparent for a person skilled in the art. A strategy starting from a
preformed nucleoside is also possible. Thus, for example, conversion of uridine
derivative 62 to derivative 44 was successfully accomplished by tosylation,
deisopropylidination and base-induced ring-closure. As another example, conversion of
nucleoside 67 into nucleoside 61B has been accomplished as depicted in Figure 34.

Conversion of the bicyclic thymine nucleoside **37** into the corresponding 5-methylcytosine nucleoside **65** was accomplished by a known reaction sequence using triazole and POCl_3 followed by benzoylation and treatment by ammonia. A similar procedure should be applicable for the synthesis of **57C** from **44**. As another example of possible strategies, coupling of precyclised furanose derivatives already containing an additional ring with nucleobase derivatives is possible. Such a strategy would in addition allow preparation of the corresponding α -nucleoside analogues. When coupling with a protected methyl furanoside of **4-C,2-O-methylene-D-ribofuranose**, we obtained mainly a ring-opened product. However, coupling of **1-O-acetyl furanose 207** or thiophenyl furanose **212** yielded successfully LNA nucleosides with the α -anomers as one product. Incorporation of such α -LNA nucleosides will be possible using the standard oligomerisation techniques (as for the LNA oligomers containing Z) yielding α -LNA oligomers. In addition, a synthetic strategy performing nucleoside coupling using a **4'-C-hydroxymethyl furanose** already activated for ring closure (e.g. by containing a mesyl or tosyl group at the **4'-C-hydroxymethyl group**), is possible as exemplified by conversion of furanose **78** to nucleoside **79** followed by deprotection and ring closure to give **36**. Chemical or enzymatic transglycosylation or anomeration of appropriate furanose derivatives or nucleosides are yet other possible synthetic strategies. These and other related strategies allow for synthesis of bicyclic nucleosides containing other nucleobases or analogues thereof by either coupling with these nucleobases or analogues, or starting from preformed nucleoside derivatives.

The described examples are meant to be illustrative for the procedures and examples of this invention. The structures of the synthesised compounds were verified using **1D** or **2D NMR** techniques, e.g. NOE experiments.

An additional embodiment of the present invention is to provide bicyclic nucleosides containing additional rings of different sizes and of different chemical structures. From the methods described it is obvious for a person skilled in the art of organic synthesis that cyclisation of other nucleosides is possible using similar procedures, also of nucleosides containing different C-branches. The person skilled in the art will be able to find inspiration and guidance for the preparation of substituted nucleoside analogue intermediates in the literature, see e.g. WO 96/14329. Regarding rings of different chemical compositions it is clear that using similar procedures or procedures well-

established in the field of organic chemistry, synthesis of for example thio analogues of the exemplified oxo analogues is possible as is the synthesis of the corresponding amino analogues (using for example nucleophilic substitution reactions or reductive alkylations).

5

In the example section, synthesis of the amino LNA analogues 73-74F are described. Conversion of 74 and 74D into standard building blocks for oligomerisation was possible by 5'-O-DMT protection and 3'-O-phosphitylation following the standard procedures. For the amino LNA analogue, protection of the 2'-amino functionality is 10 needed for controlled linear oligomerisation. Such protection can be accomplished using standard amino group protection techniques like, e.g., Fmoc, trifluoroacetyl or BOC. Alternatively, an N-alkyl group (e.g. benzyl, methyl, ethyl, propyl or functionalised alkyl) can be kept on during nucleoside transformations and oligomerisation. In Figures 35 and 36, strategies using N-trifluoroacetyl and N-methyl 15 derivatives are shown. As outlined in Figure 37, conversion of nucleoside 75 into the 2'-thio-LNA nucleoside analogue 76D has been successfully performed as has the subsequent syntheses of the phosphoramidite derivative 76F. Compound 76F has the required structure for automated synthesis of 2'-thio-LNA oligonucleotides. The N-trifluoroacetyl 2'-amino-LNA synthon 74A has the required structure for automated 20 synthesis of 2'-amino-LNA oligonucleotides.

Synthesis of the corresponding cytosine, guanine, and adenine derivatives of the 2'-thio and 2'-amino LNA nucleosides can be accomplished using strategies analogous to those shown in Figures 35, 36 and 37. Alternative, the stereochemistry around C-2' 25 can be inverted before cyclisations either by using a conveniently configurated, e.g. an arabino-configurated, furanose synthon, or by inverting the configuration around the C-2' carbon atom starting from a ribo-configurated nucleoside/furanose. Subsequent activation of the 2'- β -OH, e.g. by tosylation, double nucleophilic substitution as in the urasil/thymine example described above, could furnish the desired bicyclic 2'-thio-LNA 30 or 2'-amino-LNA nucleosides. The thus obtained properly protected cytosine, guanine, and adenine analogues can be prepared for oligomerisation using the standard reactions (DMT-protection and phosphitylation) as described above for other examples.

Preparation of oligomers

- Linear-, branched- (M. Grøtli and B. S. Sproat, *J. Chem. Soc., Chem. Commun.*, 1995, 495; R. H. E. Hudson and M. J. Damha, *J. Am. Chem. Soc.*, 1993, 115, 2119; M. Von Büren, G. V. Petersen, K. Rasmussen, G. Brandenburg, J. Wengel and F. Kirpekar, *Tetrahedron*, 1995, 51, 8491) and circular- (G. Prakash and E. T. Kool, *J. Am. Chem. Soc.*, 1992, 114, 3523) Oligo- and polynucleotides of the invention may be produced using the polymerisation techniques of nucleic acid chemistry well known to a person of ordinary skill in the art of organic chemistry. Phosphoramidite chemistry (S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1993, 49, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223) was used, but e.g. H-phosphonate chemistry, phosphortriester chemistry or enzymatic synthesis could also be used. Generally, standard coupling conditions and the phosphoramidite approach was used, but for some monomers of the invention longer coupling time, and/or repeated couplings with fresh reagents, and/or use of more concentrated coupling reagents were used. As another possibility, activators more active than 1H-tetrazole could also be used to increase the rate of the coupling reaction. The phosphoramidites 39, 46, 53, 57D, 61D, and 66 all coupled with satisfactory > 95% step-wise coupling yields. An all-phosphorothioate LNA oligomer (Table 7) was synthesised using standard procedures. Thus, by exchanging the normal, e.g. iodine/pyridine/H₂O, oxidation used for synthesis of phosphodiester oligomers with an oxidation using Beaucage's reagent (commercially available), the phosphorothioate LNA oligomer was efficiently synthesised (stepwise coupling yields > = 98%). The 2'-amino-LNA and 2'methylamino-LNA oligonucleotides (Table 9) were efficiently synthesised (step-wise coupling yields ≥ 98%) using amidites 74A and 74F. The 2'-thio-LNA oligonucleotides (Table 8) were efficiently synthesised using amidite 76F using the standard phosphoramidite procedures as described above for LNA oligonucleotides. After synthesis of the desired sequence, work up was done using standard conditions (cleavage from solid support and removal of protection groups using 30% ammonia for 55 °C for 5 h). Purification of LNA oligonucleotides was done using disposable reversed phase purification cartridges and/or reversed phase HPLC and/or precipitation from ethanol or butanol. Capillary gel electrophoresis, reversed phase HPLC and MALDI-MS was used to verify the purity of the synthesised oligonucleotide analogues,

and to verify that the desired number of bicyclic nucleoside analogues of the invention were incorporated as contemplated.

An additional aspect of the present invention is to furnish procedures for
5 oligonucleotide analogues containing LNA linked by non-natural internucleoside linkages. For example, synthesis of the corresponding phosphorothioate or phosphoramidate analogues is possible using strategies well-established in the field of oligonucleotide chemistry (*Protocols for Oligonucleotides and Analogs*, vol 20, (Sudhir Agrawal, ed.), Humana Press, 1993, Totowa, NJ; S. L. Beaucage and R. P. Iyer,
10 *Tetrahedron*, 1993, 49, 6123; S. L. Beaucage and R. P. Iyer, *Tetrahedron*, 1992, 48, 2223; E. Uhlmann and A. Peyman, *Chem. Rev.*, 90, 543).

Thus, generally the present invention also provides the use of an LNA as defined herein for the preparation of an LNA modified oligonucleotides. It should be
15 understood that LNA modified oligonucleotide may comprise normal nucleosides (i.e. naturally occurring nucleosides such as ribonucleosides and/or dioxyribonucleosides), as well as modified nucleosides different from those defined with the general formula II. In a particularly interesting embodiment, incorporation of LNA modulates the ability of the oligonucleotide to act as a substrate for nucleic acid active enzymes.
20

Furthermore, solid support materials having immobilised thereto an optionally nucleobase protected and optionally 5'-OH protected LNA are especially interesting as material for the synthesis of LNA modified oligonucleotides where an LNA monomer is included in at the 3' end. In this instance, the solid support material is preferable CPG,
25 e.g. a readily (commercially) available CPG material onto which a 3'-functionalised, optionally nucleobase protected and optionally 5'-OH protected LNA is linked using the conditions stated by the supplier for that particular material. BioGenex Universal CPG Support (BioGenex, U.S.A.) can e.g. be used. The 5'-OH protecting group may, e.g., be a DMT group. 3'-functional group should be selected with due regard to the
30 conditions applicable for the CPG material in question.

Applications

The present invention discloses the surprising finding that various novel derivatives of bicyclic nucleoside monomers (LNAs), when incorporated into oligonucleotides,

- 5 dramatically increase the affinity of these modified oligonucleotides for both complementary ssDNA and ssRNA compared to the unmodified oligonucleotides. It further discloses the surprising finding that both fully and partly LNA modified oligonucleotides display greatly enhanced hybridisation properties for their complementary nucleic acid sequences. Depending on the application, the use of these
- 10 LNAs thus offers the intriguing possibility to either greatly increase the affinity of a standard oligonucleotide without compromising specificity (constant size of oligonucleotide) or significantly increase the specificity without compromising affinity (reduction in the size of the oligonucleotide). The present invention also discloses the unexpected finding that LNA modified oligonucleotides, in addition to greatly enhanced
- 15 hybridisation properties, display many of the useful physicochemical properties of normal DNA and RNA oligonucleotides. Examples given herein include excellent solubility, a response of LNA modified oligonucleotides to salts like sodium chloride and tetramethylammonium chloride which mimic that of the unmodified oligonucleotides, the ability of LNA modified oligonucleotides to act as primers for a
- 20 variety of polymerases, the ability of LNA modified nucleotides to act as primers in a target amplification reaction using a thermostable DNA polymerase, the ability of LNA modified oligonucleotides to act as a substrate for T4 polynucleotide kinase, the ability of biotinylated LNAs to sequence specifically capture PCR amplicons onto a streptavidine coated solid surface, the ability of immobilised LNA modified
- 25 oligonucleotides to sequence specifically capture amplicons and very importantly the ability of LNA modified oligonucleotides to sequence specifically target double-stranded DNA by strand invasion. Hence, it is apparent to one of ordinary skills in the art that these novel nucleoside analogues are extremely useful tools to improve the performance in general of oligonucleotide based techniques in therapeutics,
- 30 diagnostics and molecular biology.

An object of the present invention is to provide monomeric LNAs according to the invention which can be incorporated into oligonucleotides using procedures and equipment well known to one skilled in the art of oligonucleotide synthesis.

Another object of the present invention is to provide fully or partly LNA modified oligonucleotides (oligomers) that are able to hybridise in a sequence specific manner to complementary oligonucleotides forming either duplexes or triplexes of substantially 5 higher affinity than the corresponding complexes formed by unmodified oligonucleotides.

Another object of the present invention is to use LNAs to enhance the specificity of normal oligonucleotides without compromising affinity. This can be achieved by 10 reducing the size (and therefore affinity) of the normal oligonucleotide to an extent that equals the gain in affinity resulting from the incorporation of LNAs.

Another object of the present invention is to provide fully or partly modified oligonucleotides containing both LNAs, normal nucleosides and other nucleoside 15 analogues.

A further object of the present invention is to exploit the high affinity of LNAs to create modified oligonucleotides of extreme affinity that are capable of binding to their target sequences in a dsDNA molecule by way of "strand displacement".

20 A further object of the invention is to provide different classes of LNAs which, when incorporated into oligonucleotides, differ in their affinity towards their complementary nucleosides. In accordance with the invention this can be achieved by either substituting the normal nucleobases G, A, T, C and U with derivatives having, for 25 example, altered hydrogen bonding possibilities or by using LNAs that differ in their backbone structure. The availability of such different LNAs facilitates exquisite tuning of the affinity of modified oligonucleotides.

Another object of the present invention is to provide LNA modified oligonucleotides 30 which are more resistant to nucleases than their unmodified counterparts.

Another object of the present invention is to provide LNA modified oligonucleotides which can recruit RNaseH.

An additional object of the present invention is to provide LNAs that can act as substrates for DNA and RNA polymerases thereby allowing the analogues to be either incorporated into a growing nucleic acid chain or to act as chain terminators.

- 5 A further object of the present invention is to provide LNAs that can act as therapeutic agents. Many examples of therapeutic nucleoside analogues are known and similar derivatives of the nucleoside analogues disclosed herein can be synthesised using the procedures known from the literature (E. De Clercq, *J. Med. Chem.* 1995, 38, 2491; P. Herdewijn and E. De Clercq: Classical Antiviral Agents and
- 10 Design og New Antiviral Agents. In: *A Textbook of Drug Design and Development*; Eds. P. Krogsgaard-Larsen, T. Liljefors and U. Madsen; Harwood Academic Publishers, Amsterdam, 1996, p. 425; I. K. Larsen: Anticancer Agents. In: *A Textbook of Drug Design and Development*; Eds. P. Krogsgaard-Larsen, T. Liljefors and U. Madsen; Harwood Academic Publishers, Amsterdam, 1996, p. 460).

15

- Double-stranded RNA has been demonstrated to posses anti-viral activity and tumour suppressing activity (Sharp et al., *Eur. J. Biochem.* 230(1): 97-103, 1995, Lengyel-P. et al., *Proc. Natl. Acad. Sci. U.S.A.*, 90(13): 5893-5, 1993, and Laurent-Crawford et al., *AIDS Res. Hum. Retroviruses*, 8(2): 285-90, 1992). It is likely that double stranded LNAs may mimic the effect of therapeutically active double stranded RNAs and accordingly such double stranded LNAs has a potential as therapeutic drugs.

- When used herein, the term "natural nucleic acid" refers to nucleic acids in the broadest sense, like for instance nucleic acids present in intact cells of any origin or
- 25 vira or nucleic acids released from such sources by chemical or physical means or nucleic acids derived from such primary sources by way of amplification. The natural nucleic acid may be single, double or partly double stranded, and may be a relatively pure species or a mixture of different nucleic acids. It may also be a component of a crude biological sample containing other nucleic acids and other cellular components.
 - 30 On the other hand, the term "synthetic nucleic acids" refers to any nucleic acid produced by chemical synthesis.

The present invention also provides the use of LNA modified oligonucleotides in nucleic acid based therapeutic, diagnostics and molecular biology. The LNA modified oligonucleotides can be used in the detection, identification, capture, characterisation, quantification and fragmentation of natural or synthetic nucleic acids, and as blocking

- 5 agents for translation and transcription *in vivo* and *in vitro*. In many cases it will be of interest to attach various molecules to LNA modified oligonucleotides. Such molecules may be attached to either end of the oligonucleotide or they may be attached at one or more internal positions. Alternatively, they may be attached to the oligonucleotide via spacers attached to the 5' or 3' end. Representative groups of such molecules are
- 10 DNA intercalators, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands. Generally all methods for labelling unmodified DNA and RNA oligonucleotides with these molecules can also be used to label LNA modified oligonucleotides. Likewise, all methods used for detecting labelled oligonucleotides generally apply to the corresponding labelled, LNA modified
- 15 oligonucleotides.

Therapy

The term "strand displacement" relates to a process whereby an oligonucleotide binds

- 20 to its complementary target sequence in a double stranded DNA or RNA so as to displace the other strand from said target strand.

In an aspect of the present invention, LNA modified oligonucleotides capable of performing "strand displacement" are exploited in the development of novel

- 25 pharmaceutical drugs based on the "antigene" approach. In contrast to oligonucleotides capable of making triple helices, such "strand displacement" oligonucleotides allow any sequence in a dsDNA to be targeted and at physiological ionic strength and pH.

- 30 The "strand displacing" oligonucleotides can also be used advantageously in the antisense approach in cases where the RNA target sequence is inaccessible due to intramolecular hydrogen bonds. Such intramolecular structures may occur in mRNAs and can cause significant problems when attempting to "shut down" the translation of the mRNA by the antisense approach.

Other classes of cellular RNAs, like for instance tRNAs, rRNAs snRNAs and scRNAs, contain intramolecular structures that are important for their function. These classes of highly structured RNAs do not encode proteins but rather (in the form of

- 5 RNA/protein particles) participate in a range of cellular functions such as mRNA splicing, polyadenylation, translation, editing, maintainance of chromosome end integrity, etc.. Due to their high degree of structure, that impairs or even prevent normal oligonucleotides from hybridising efficiently, these classes of RNAs has so far not attracted interest as antisense targets.

10

The use of high affinity LNA monomers should facilitate the construction of antisense probes of sufficient thermostability to hybridise effectively to such target RNAs.

Therefore, in a preferred embodiment, LNA is used to confer sufficient affinity to the oligonucleotide to allow it to hybridise to these RNA classes thereby modulating the

15 qualitative and/or quantitative function of the particles in which the RNAs are found.

In some cases it may be advantageous to down-regulate the expression of a gene whereas in other cases it may be advantageous to activate it. As shown by Møllegaard et al. (Møllegaard, N. E.; Buchardt, O.; Egholm, M.; Nielsen, P. E. *Proc.*

20 *Natl. Acad. Sci. U.S.A.* 1994, 91, 3892), oligomers capable of "strand displacement" can function as RNA transcriptional activators. In an aspect of the present invention, the LNAs capable of "strand displacement" are used to activate genes of therapeutic interest.

25 In chemotherapy of numerous viral infections and cancers, nucleosides and nucleoside analogues have proven effective. LNA nucleosides are potentially useful as such nucleoside based drugs.

Various types of double-stranded RNAs inhibit the growth of several types of cancers.

30 Duplexes involving one or more LNA oligonucleotide(s) are potentially useful as such double-stranded drugs.

The invention also concerns a pharmaceutical composition comprising a pharmaceutically active LNA modified oligonucleotide or a pharmaceutically active

LNA monomer as defined above in combination with a pharmaceutically acceptable carrier.

Such compositions may be in a form adapted to oral, parenteral (intravenous, 5 intraperitoneal), intramuscular, rectal, intranasal, dermal, vaginal, buccal, ocularly, or pulmonary administration, preferably in a form adapted to oral administration, and such compositions may be prepared in a manner well-known to the person skilled in the art, e.g. as generally described in "Remington's Pharmaceutical Sciences", 17. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, U.S.A., 1985 and 10 more recent editions and in the monographs in the "Drugs and the Pharmaceutical Sciences" series, Marcel Dekker.

Diagnostics

15 Several diagnostic and molecular biology procedures have been developed that utilise panels of different oligonucleotides to simultaneously analyse a target nucleic acid for the presence of a plethora of possible mutations. Typically, the oligonucleotide panels are immobilised in a predetermined pattern on a solid support such that the presence of a particular mutation in the target nucleic acid can be revealed by the position on 20 the solid support where it hybridises. One important prerequisite for the successful use of panels of different oligonucleotides in the analysis of nucleic acids is that they are all specific for their particular target sequence under the single applied hybridisation condition. Since the affinity and specificity of standard oligonucleotides for their complementary target sequences depend heavily on their sequence and size this 25 criteria has been difficult to fulfil so far.

In a preferred embodiment, therefore, LNAs are used as a means to increase affinity and/or specificity of the probes and as a means to equalise the affinity of different oligonucleotides for their complementary sequences. As disclosed herein such affinity 30 modulation can be accomplished by, e.g., replacing selected nucleosides in the oligonucleotide with an LNA carrying a similar nucleobase. As further shown herein, different classes of LNAs exhibit different affinities for their complementary nucleosides. For instance, the 2-3 bridged LNA with the T-nucleobase exhibits less affinity for the A-nucleoside than the corresponding 2-4 bridged LNA. Hence, the use

of different classes of LNAs offers an additional level for fine-tuning the affinity of a oligonucleotide.

In another preferred embodiment the high affinity and specificity of LNA modified

- 5 oligonucleotides is exploited in the sequence specific capture and purification of natural or synthetic nucleic acids. In one aspect, the natural or synthetic nucleic acids are contacted with the LNA modified oligonucleotide immobilised on a solid surface. In this case hybridisation and capture occurs simultaneously. The captured nucleic acids may be, for instance, detected, characterised, quantified or amplified directly on the
- 10 surface by a variety of methods well known in the art or it may be released from the surface, before such characterisation or amplification occurs, by subjecting the immobilised, modified oligonucleotide and captured nucleic acid to dehybridising conditions, such as for example heat or by using buffers of low ionic strength.

- 15 The solid support may be chosen from a wide range of polymer materials such as for instance CPG (controlled pore glass), polypropylene, polystyrene, polycarbonate or polyethylene and it may take a variety of forms such as for instance a tube, a micro-titer plate, a stick, a bead, a filter, etc.. The LNA modified oligonucleotide may be immobilised to the solid support via its 5' or 3' end (or via the terminus of linkers
- 20 attached to the 5' or 3' end) by a variety of chemical or photochemical methods usually employed in the immobilisation of oligonucleotides or by non-covalent coupling such as for instance via binding of a biotinylated LNA modified oligonucleotide to immobilised streptavidin. One preferred method for immobilising LNA modified oligonucleotides on different solid supports is photochemical using a photochemically
- 25 active anthraquinone covalently attached to the 5' or 3' end of the modified oligonucleotide (optionally via linkers) as described in (WO 96/31557). Thus, the present invention also provide a surface carrying an LNA modified oligonucleotide.

In another aspect the LNA modified oligonucleotide carries a ligand covalently

- 30 attached to either the 5' or 3' end. In this case the LNA modified oligonucleotide is contacted with the natural or synthetic nucleic acids in solution whereafter the hybrids formed are captured onto a solid support carrying molecules that can specifically bind the ligand.

In still another aspect, LNA modified oligonucleotides capable of performing "strand displacement" are used in the capture of natural and synthetic nucleic acids without prior denaturation. Such modified oligonucleotides are particularly useful in cases where the target sequence is difficult or impossible to access by normal

5 oligonucleotides due to the rapid formation of stable intramolecular structures.

Examples of nucleic acids containing such structures are rRNA, tRNA, snRNA and scRNA.

In another preferred embodiment, LNA modified oligonucleotides designed with the

10 purpose of high specificity are used as primers in the sequencing of nucleic acids and as primers in any of the several well known amplification reactions, such as the PCR reaction. As shown herein, the design of the LNA modified oligonucleotides determines whether it will sustain a exponential or linear target amplification. The

products of the amplification reaction can be analysed by a variety of methods

15 applicable to the analysis of amplification products generated with normal DNA primers. In the particular case where the LNA modified oligonucleotide primers are

designed to sustain a linear amplification the resulting amplicons will carry single stranded ends that can be targeted by complementary probes without denaturation. Such ends could for instance be used to capture amplicons by other complementary

20 LNA modified oligonucleotides attached to a solid surface.

In another aspect, LNA modified oligos capable of "strand displacement" are used as primers in either linear or exponential amplification reactions. The use of such oligos is expected to enhance overall amplicon yields by effectively competing with amplicon

25 re-hybridisation in the later stages of the amplification reaction. Demers, et al. (Nucl.

Acid Res. 1995, Vol 23, 3050-3055) discloses the use of high-affinity, non-extendible oligos as a means of increasing the overall yield of a PCR reaction. It is believed that the oligomers elicit these effect by interfering with amplicon re-hybridisation in the later stages of the PCR reaction. It is expected that LNA modified oligos blocked at

30 their 3' end will provide the same advantage. Blocking of the 3' end can be achieved in numerous ways like for instance by exchanging the 3' hydroxyl group with

hydrogen or phosphate. Such 3' blocked LNA modified oligos can also be used to selectively amplify closely related nucleic acid sequences in a way similar to that described by Yu et al. (Biotechniques, 1997, 23, 714-716).

In recent years, novel classes of probes that can be used in for example real-time detection of amplicons generated by target amplification reactions have been invented.

One such class of probes have been termed "Molecular Beacons". These probes are

- 5 synthesised as partly self-complementary oligonucleotides containing a fluorophor at one end and a quencher molecule at the other end. When free in solution the probe folds up into a hairpin structure (guided by the self-complementary regions) which positions the quencher in sufficient closeness to the fluorophor to quench its fluorescent signal. Upon hybridisation to its target nucleic acid, the hairpin opens
- 10 thereby separating the fluorophor and quencher and giving off a fluorescent signal.

Another class of probes have been termed "Taqman probes". These probes also

contain a fluorophor and a quencher molecule. Contrary to the Molecular Beacons, however, the quencher's ability to quench the fluorescent signal from the fluorophor is

- 15 maintained after hybridisation of the probe to its target sequence. Instead, the fluorescent signal is generated after hybridisation by physical detachment of either the quencher or fluorophor from the probe by the action of the 5' exonuclease activity of a polymerase which has initiated synthesis from a primer located 5' to the binding site of the Taqman probe.

- 20 High affinity for the target site is an important feature in both types of probes and consequently such probes tend to be fairly large (typically 30 to 40 mers). As a result, significant problems are encountered in the production of high quality probes. In a preferred embodiment, therefore, LNA is used to improve production and subsequent performance of Taqman probes and Molecular Beacons by reducing their
- 25 size whilst retaining the required affinity.

In a further aspect, LNAs are used to construct new affinity pairs (either fully or partially modified oligonucleotides). The affinity constants can easily be adjusted over a wide range and a vast number of affinity pairs can be designed and synthesised.

- 30 One part of the affinity pair can be attached to the molecule of interest (e.g. proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, peptides, PNA, etc.) by standard methods, while the other part of the affinity pair can be attached to e.g. a solid support such as beads, membranes, micro-titer plates, sticks, tubes, etc. The solid support may be chosen from a wide range of polymer materials such as for

instance polypropylene, polystyrene, polycarbonate or polyethylene. The affinity pairs may be used in selective isolation, purification, capture and detection of a diversity of the target molecules mentioned above.

- 5 The principle of capturing an LNA-tagged molecule by ways of interaction with another complementary LNA oligonucleotide (either fully or partially modified) can be used to create an infinite number of novel affinity pairs.

- In another preferred embodiment the high affinity and specificity of LNA modified oligonucleotides are exploited in the construction of probes useful in *in-situ* hybridisation. For instance, LNA could be used to reduce the size of traditional DNA probes whilst maintaining the required affinity thereby increasing the kinetics of the probe and its ability to penetrate the sample specimen. The ability of LNA modified oligonucleotides to "strand invade" double stranded nucleic acid structures are also of considerable advantage in *in-situ* hybridisation, because it facilitates hybridisation without prior denaturation of the target DNA/RNA.

- In another preferred embodiment, LNA modified oligonucleotides to be used in antisense therapeutics are designed with the dual purpose of high affinity and ability to recruit RNaseH. This can be achieved by, for instance, having LNA segments flanking an unmodified central DNA segment.

- The present invention also provides a kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, where the kit comprises a reaction body and one or more LNA modified oligonucleotides (oligomer) as defined herein. The LNA modified oligonucleotides are preferably immobilised onto said reactions body.

- The present invention also provides a kit for the isolation, purification, amplification, detection, identification, quantification, or capture of natural or synthetic nucleic acids, where the kit comprises a reaction body and one or more LNAs as defined herein. The LNAs are preferably immobilised onto said reactions body (e.g. by using the immobilising techniques described above).

- For the kits according to the invention, the reaction body is preferably a solid support material, e.g. selected from borosilicate glass, soda-lime glass, polystyrene, polycarbonate, polypropylene, polyethylene, polyethyleneglycol terephthalate, polyvinylacetate, polyvinylpyrrolidinone, polymethylmethacrylate and polyvinylchloride,
- 5 preferably polystyrene and polycarbonate. The reaction body may be in the form of a specimen tube, a vial, a slide, a sheet, a film, a bead, a pellet, a disc, a plate, a ring, a rod, a net, a filter, a tray, a microtitre plate, a stick, or a multi-bladed stick.
- 10 The kits are typically accompanied by a written instruction sheet stating the optimal conditions for use of the kit.

The above-mentioned diagnostic and therapeutic aspects of the present invention have
15 been illustrated with the following examples.

EXPERIMENTAL**General**

- 5 All reagents were obtained from commercial suppliers and were used without further purification. After drying any organic phase using Na_2SO_4 , filtration was performed. The silica gel (0.040-0.063 mm) used for column chromatography was purchased from Merck. NMR spectra were recorded at 300 MHz or 250 MHz for ^1H NMR and 62.9 MHz for ^{13}C NMR and at 202.33 MHz for ^{31}P NMR. δ -Values are in ppm relative
10 to tetramethylsilane as internal standard (^1H NMR and ^{13}C NMR) and relative to 85% H_3PO_4 as external standard (^{31}P NMR). Assignments of NMR peaks are given according to standard nucleoside nomenclature. EI mass spectra, FAB mass spectra and Plasma Desorption mass spectra were recorded to gain information on the molecular weight of synthesised compounds. Oligonucleotide analogues were synthesised using the
15 phosphoramidite methodology. Purification of 5'-O-DMT-ON or 5'-O-DMT-OFF oligonucleotide analogues was accomplished using disposable reversed phase chromatography cartridges or reversed phase HPLC when necessary. Matrix-assisted laser desorption mass spectra were obtained to verify the molecular weight and monomer composition of representative oligonucleotide samples. Capillary gel
20 electrophoresis was performed to verify the purity of representative oligonucleotide samples.

The specific descriptions below are accompanied by Figures 2-41 and Tables 1-10.

Unless otherwise stated in the following examples, "LNA" designates the 2'-4'-bridged

- 25 variant illustrated with the formula Z in Figure 2.

Preparation of LNA monomers**Example 1**

- 30 3-C-Allyl-1,2-O-isopropylidene- α -D-ribofuranose (OA). *Method 1:* A solution of 5-O-t-butylidemethylsilyl-1,2-O-isopropylidene- α -D-ribofuran-3-ulose (Y. Yoshimura, T. Sano, A. Matsuda and T. Ueda, *Chem. Pharm. Bull.*, 1988, **36**, 162) (17.8 g, 58.9 mmol) in anhydrous THF (980 cm³) was stirred at 0 °C and 1 M allylmagnesium bromide in

anhydrous ether (130 cm³, 130 mmol) was added dropwise. After stirring for 2 h, a saturated aqueous solution of ammonium chloride (800 cm³) was added and the mixture was extracted with dichloromethane (3 x 400 cm³). The organic phase was washed with brine (3 x 450 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was dissolved in anhydrous THF (700 cm³). A 1.1 M solution of tetrabutylammonium fluoride in THF (54.4 cm³, 59.8 mmol) was added and the mixture was stirred at room temperature for 1 h and evaporated to dryness. The residue was dissolved in dichloromethane (1700 cm³) and was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 500 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give furanose 0A as a white solid material (9.42 g, 69%). *Method 2:* Furanose 0A was analogously synthesised from 5-O-t-butylidiphenylsilyl-1,2-O-isopropylidene- α -D-ribofuran-3-ulose (T. F. Tam and B. Fraser-Reid, *J. Chem. Soc., Chem. Commun.*, 1980, 556) (9.5 g, 22.2 mmol) using: anhydrous THF (425 cm³); a 1 M solution of allylmagnesium bromide in anhydrous ether (130 cm³, 130 mmol); a saturated aqueous solution of ammonium chloride (490 cm³); ether for extraction (350 + 2 x 160 cm³); brine (2 x 160 cm³); a 1.1 M solution of tetrabutylammonium fluoride in THF (22.3 cm³, 24.6 mmol); anhydrous THF (400 cm³); dichloromethane (1400 cm³); a saturated aqueous solution of sodium hydrogencarbonate (3 x 500 cm³); brine (500 cm³) and (Na₂SO₄). δ_H ((CD₃)₂SO) 5.84 (1 H, m, 2'-H), 5.65 (1 H, d, J 3.8, 1-H), 5.12 (1 H, d, J 6.1, 3'-H_a), 5.06 (1 H, br s, 3'-H_b), 4.76 (1 H, s, 3-OH), 4.64 (1 H, t, J 5.4, 5-OH), 4.16 (1 H, d, J 3.8, 2-H), 3.84 (1 H, dd, J 2.2, 8.1, 4-H), 3.56 (1 H, ddd, J 2.3, 5.6, 11.8, 5-H_a), 3.42 (1 H, m, 5-H_b), 2.16 (1 H, dd, J 6.1, 14.3, 1'-H_a), 1.98 (1 H, dd, J 8.2, 14.3, 1'-H_b), 1.46 (3 H, s, CH₃), 1.25 (3 H, s, CH₃). δ_C (CDCl₃) 133.5 (C-2'), 117.9 (C-3'), 110.8 (C(CH₃)₂), 102.9 (C-1), 82.6, 81.0, 77.7 (C-2, C-3, C-4), 59.4 (C-5), 36.4 (C-1'), 26.4, 26.3 (CH₃) (Found: C, 57.4; H, 8.0; C₁₁H₁₈O₅ requires C, 57.4; H, 7.9%).

Example 2

3-C-Allyl-3,5-di-O-benzyl-1,2-O-isopropylidene- α -D-ribofuranose (OB). A 60%

suspension of sodium hydride (4.9 g, 123 mmol) in anhydrous DMF (100 cm³) was

stirred at 0 °C and a solution of furanose **OA** (9.42 g, 40.9 mmol) in anhydrous DMF

(65 cm³) was added dropwise over 45 min. The solution was stirred for 1 h at 50 °C and cooled to 0 °C. A mixture of benzyl bromide (14.5 cm³, 121 mmol) and anhydrous DMF (14.5 cm³) was added dropwise and the mixture was stirred at room temperature for 18 h. The reaction mixture was evaporated to dryness and a solution of the residue in dichloromethane (700 cm³) was washed with a saturated aqueous solution of sodium hydrogencarbonate (2 x 450 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using petroleum ether/ethylacetate (9:1, v/v) as eluent to give compound **OB** as an oil (14.5 g, 86%). δ_H (CDCl₃) 7.39-7.21 (10H, m, Bn), 5.92 (1 H, m, 2'-H), 5.71 (1 H, d, J 3.8, 1-H), 5.17-5.09 (2 H, m, 3'-H_a, 3'-H_b), 4.67 (2 H, m, Bn), 4.60 (1 H, d, J 12.2, Bn), 4.52 (1 H, d, J 12.1, Bn), 4.43 (1 H, m, 4-H), 4.42 (1 H, d, J 3.8, 2-H), 3.73 (1 H, dd, J 3.2, 10.8, 5-H_a), 3.66 (1 H, dd, J 7.4, 10.8, 5-H_b), 2.50 (1 H, dd, J 7.7, 14.9, 1'-H_a), 2.39 (1 H, dd, J 6.5, 14.9, 1'-H_b), 1.60 (3 H, s, CH₃), 1.34 (3 H, s, CH₃). δ_C (CDCl₃) 138.7, 138.1 (Bn), 132.6 (C-2'), 128.3, 128.2, 127.7, 127.5, 127.4, 127.4 (Bn), 118.5 (C-3'), 112.6 (C(CH₃)₂), 104.1 (C-1), 86.5, 82.1, 80.4 (C-2, C-3, C-4), 73.4, 68.6 (Bn), 67.0 (C-5), 35.8 (C-1'), 26.8, 26.6 (CH₃). FAB-MS *m/z* 433 [M + Na]⁺ (Found: C, 73.4; H, 7.4; C₂₅H₃₀O₅ requires C, 73.2; H, 7.4%).

Example 3

25 3-C-Allyl-1,2-di-O-acetyl-3,5-di-O-benzyl-D-ribofuranose (OC). A solution of furanose **OB** (12.42 g, 30.3 mmol) in 80% aqueous acetic acid (150 cm³) was stirred at 90 °C for 3 h. The solvent was removed under reduced pressure and the residue was coevaporated with ethanol (3 x 75 cm³), toluene (3 x 75 cm³) and anhydrous pyridine (2 x 75 cm³) and redissolved in anhydrous pyridine (60 cm³). Acetic anhydride (46 cm³) was added and the solution was stirred at room temperature for 48 h. A mixture of ice and water (300 cm³) was added and the resulting mixture was extracted with dichloromethane (2 x 300 cm³). The combined organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 200 cm³) and dried (Na₂SO₄). The solvent was evaporated and the residue was purified using silica gel

column chromatography with petroleum ether/ethyl acetate (4:1, v/v) as eluent to give the anomeric mixture **0C** ($\beta:\alpha \sim 2:1$) as an oil (13.3 g, 97%). δ_c (CDCl₃) 169.7, 169.6 (C=O), 138.7, 138.4, 137.7, 137.6 (Bn), 132.4, 132.2 (C-2'), 128.4 128.4, 128.2, 128.2, 127.8, 127.7, 127.7, 127.6, 127.3, 127.3, 126.9, 126.8 (Bn), 118.5 (C-3'), 5 99.4, 93.5 (C-1), 84.8, 83.7, 83.2, 82.0, 79.1, 75.5 (C-2, C-3, C-4), 73.7, 73.5, 69.3, 68.7 (Bn), 66.1 (C-5), 35.5, 34.9 (C-1), 21.1, 21.0, 20.7, 20.6 (CH₃) (Found: C, 68.7; H, 6.7; C₂₆H₃₀O, requires C, 68.8; H, 6.6%).

Example 4

- 10 **1-(2-O-Acetyl-3-C-allyl-3,5-di-O-benzyl- β -D-ribofuranosyl)thymine (1).** To a stirred solution of the anomeric mixture **0C** ($\beta:\alpha \sim 2:1$, 11.8 g, 26.0 mmol) (P. Nielsen, H. M. Pfundheller and J. Wengel, *Chem. Commun.*, 1997, 825; P. Nielsen, H. M. Pfundheller, C. E. Olsen and J. Wengel, *J. Chem. Soc., Perkin Trans. 1*, 1997, in the press) and thymine (6.55 g, 52.0 mmol) in anhydrous acetonitrile (250 cm³) was 15 added *N,O*-bis(trimethylsilyl)acetamide (44.9 cm³, 182 mmol). The reaction mixture was stirred at reflux for 1 h and cooled to 0 °C. Trimethylsilyl triflate (8.00 cm³, 44.0 mmol) was added dropwise and the solution was stirred at room temperature for 12 h. An ice-cold saturated aqueous solution of sodium hydrogencarbonate (270 cm³) was added and the mixture was extracted with dichloromethane (3 x 125 cm³). The 20 organic phase was washed with saturated aqueous solutions of sodium hydrogen-carbonate (2 x 125 cm³) and brine (2 x 125 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give nucleoside **1** as a white solid material (11.6 g, 86%). δ_h (CDCl₃) 8.64 (1 H, br s, NH), 25 7.75 (1 H, d, *J* 1.1, 6-H), 7.41-7.25 (10 H, m, Bn), 6.43 (1 H, d, *J* 8.2, 1'-H), 5.88 (1 H, m, 2''-H), 5.66 (1 H, d, *J* 8.2, 2'-H), 5.12 (1 H, s, 3''-H_a), 5.07 (1 H, dd, *J* 1.5, 8.5, 3''-H_b), 4.85 (1 H, d, *J* 11.2, Bn), 4.64 (2 H, s, Bn), 4.63 (1 H, d, *J* 11.2, Bn), 4.33 (1 H, br s, 4'-H), 3.81 (1 H, dd, *J* 2.7, 11.1, 5'-H_a), 3.65 (1 H, m, 5'-H_b), 2.81-2.65 (2 H, m, 1''-H_a, 1''-H_b), 2.08 (3 H, s, COCH₃), 1.52 (3 H, d, *J* 0.8, CH₃). δ_c 30 (CDCl₃) 170.1 (C=O), 163.6 (C-4), 150.9 (C-2), 138.1, 136.6 (Bn), 136.0 (C-6), 131.6 (C-2''), 128.8, 128.4, 128.3, 127.6, 127.5, 127.1 (Bn), 118.5 (C-3''), 111.1 (C-5), 84.2, 83.4, 83.1, 77.4 (C-1', C-2', C-3', C-4'), 73.6, 69.2 (Bn), 65.6 (C-5'), 33.7 (C-1''), 20.8 (COCH₃), 11.9 (CH₃) (Found: C, 66.8; H, 6.3; N, 5.1. C₂₉H₃₂N₂O₇ requires C, 66.9; H, 6.2; N, 5.4%).

Example 5

1-(3-C-Allyl-3,5-di-O-benzyl- β -D-ribofuranosyl)thymine (2). To a stirred solution of nucleoside 1 (11.6 g, 22.3 mmol) in methanol (110 cm³) was added sodium 5 methoxide (3.03 g, 55.5 mmol). The reaction mixture was stirred at room temperature for 16 h and neutralised with dilute hydrochloric acid. The solvent was partly evaporated and the residue was dissolved in dichloromethane (2 x 400 cm³). The organic phase was washed with a saturated aqueous solution of sodium hydrogen-carbonate (3 x 250 cm³) and dried (Na₂SO₄). The solvent was removed under reduced 10 pressure to give 2 as a white solid material (10.1 g, 95%). δ_H (CDCl₃) 8.77 (1 H, br s, NH), 7.58 (1 H, d, J 1.2, 6-H), 7.41-7.25 (10 H, m, Bn), 6.14 (1H, m, 2''-H), 6.12 (1 H, d, J 7.8, 1'-H), 5.23 (1 H, m, 3''-H_a), 5.17 (1 H, br s, 3''-H_b), 4.68 (1 H, d, J 10.8, Bn), 4.59 (2 H, s, Bn), 4.55 (1 H, d, J 10.9, Bn), 4.39 (1 H, br s, 4'-H), 4.26 (1 H, dd J 7.8, 10.7, 2'-H), 3.84 (1 H, dd, J 3.1, 11.0, 5'-H_a), 3.58 (1 H, dd, J 1.4, 11.0, 5'-H_b), 3.04 (1 H, d, J 10.8, 2'-OH), 2.82-2.78 (2 H, m, 1''-H_a, 1''-H_b), 1.51 (3 H, d, J 1.0, CH₃). δ_C (CDCl₃) 163.5 (C-4), 151.1 (C-2), 137.3, 136.7 (Bn), 136.0 (C-6), 132.1 (C-2''), 128.8, 128.5, 128.3, 127.9, 127.6 (Bn), 118.4 (C-3''), 111.1 (C-5), 87.4, 82.6, 81.1, 79.3 (C-1', C-2', C-3', C-4'), 73.7, 69.8 (Bn), 64.7 (C-5'), 35.1 (C-1''), 11.9 (CH₃). (Found: C, 67.8; H, 6.1; N, 5.5. C₂₇H₃₀N₂O₆ requires C, 67.8; H, 6.3; N, 5.9%).

Example 6

1-(3-C-Allyl-3,5-di-O-benzyl-2-O-methanesulfonyl- β -D-ribofuranosyl)thymine (3). To a stirred solution of nucleoside 2 (3.50 g, 7.31 mmol) in anhydrous pyridine (23 cm³) at 25 0 °C was added methanesulphonyl chloride (1.69 cm³, 21.89 mmol). The reaction mixture was stirred for 1 h at room temperature, water (100 cm³) was added and extraction was performed using dichloromethane (3 x 150 cm³). The organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 200 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the 30 residue purified by silica gel column chromatography using dichloromethane/methanol (99:1) as eluent to give 3 as a white solid material (3.64 g, 89%). δ_H (CDCl₃) 8.95 (1 H, br s, NH), 7.71 (1 H, d, J 1.1, 6-H), 7.39-7.25 (10 H, m, Bn), 6.52 (1 H, d, J 8.0, 1'-H), 5.90 (1H, m, 2''-H), 5.34 (1 H, d, J 7.9, 2'-H), 5.20-5.09 (2 H, m, 3''-H_a, 3''-H_b), 4.91 (1 H, d, J 11.2, Bn), 4.68 (1 H, d, J 11.3, Bn), 4.64 (2 H, s, Bn), 4.33

(1 H, br s, 4'-H), 3.81 (1 H, dd, *J* 2.5, 11.1, 5'-H_a), 3.73 (1 H, dd, *J* 1.1, 11.1, 5'-H_b), 3.08 (1 H, dd, *J* 5.5, 5.7, 1''-H_a), 2.99 (3 H, s, CH₃), 2.68 (1 H, m, 1''-H_b), 1.51 (3 H, d, *J* 0.8, CH₃). δ_C (CDCl₃) 163.4 (C-4), 150.8 (C-2), 137.9, 136.3 (Bn), 135.5 (C-6), 131.0 (C-2''), 128.8, 128.3, 127.5, 127.2 (Bn), 119.3 (C-3''), 111.6 (C-5), 84.1, 83.6, 82.4, 82.2 (C-1', C-2', C-3', C-4'), 73.7, 68.9 (Bn), 66.2 (C-5'), 38.7 (CH₃), 33.0 (C-1''). 11.9 (CH₃) (Found: C, 60.5; H, 5.8; N, 4.9. C₂₈H₃₂N₂O₆S requires C, 60.4; H, 5.8; N, 5.0%).

Example 7

- 10 **1-(3-C-Allyl-3,5-di-O-benzyl-β-D-arabinofuranosyl)thymine (4).** A solution of nucleoside 3 (3.59 g, 6.45 mmol) in ethanol (72 cm³), water (72 cm³) and 1 M aqueous sodium hydroxide (20.6 cm³) was stirred under reflux for 18 h. After neutralisation with dilute hydrochloric acid, the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (3 x 150 cm³). The organic phase was washed with 15 a saturated aqueous solution of sodium hydrogencarbonate (3 x 200 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give 4 as a white solid material (2.32 g, 74%). δ_H (CDCl₃) 7.60 (1 H, d, *J* 1.2, 6-H), 7.50-7.23 (10 H, m, Bn), 6.22 (1 H, d, *J* 2.9, 1'-H), 5.80 (1 H, m, 2''-H), 5.15-5.08 (2 H, m, 3''-H_a, 3''-H_b), 4.86-4.33 (6 H, m, 2 x Bn, 2'-H, 4'-H), 3.82-3.71 (2 H, m, 5'-H_a, 5'-H_b), 2.72 (1 H, m, 1''-H_a), 2.52 (1 H, dd, *J* 7.6, 16.1, 1''-H_b), 1.70 (3 H, d, *J* 0.9, CH₃). δ_C (CDCl₃) 165.1 (C-4), 150.4 (C-2), 138.4, 136.8 (Bn), 137.7 (C-6), 132.3 (C-2''), 128.77 128.4, 128.3, 128.0, 127.9, 127.6 (Bn), 118.5, (C-3''), 107.8 (C-5), 88.0, 87.8, 83.7 (C-1', C-3', C-4'), 73.7, 72.9, 69.4 (Bn, C-2'), 25 64.7 (C-5'), 31.1 (C-1''), 12.4 (CH₃) (Found: C, 67.5; H, 6.3; N, 5.3). C₂₇H₃₀N₂O₆.0.25H₂O requires C, 67.1; H, 6.4; N, 5.8%).

Example 8

- 1-(3,5-Di-O-benzyl-3-C-(2-hydroxyethyl)-β-D-arabinofuranosyl)thymine (5).** To a stirred 30 solution of nucleoside 4 (2.26 g, 4.68 mmol) in THF (12 cm³) and water (12 cm³) was added sodium periodate (3.04 g, 14.2 mmol) and a 2.5% solution of osmium tetroxide in *tert*-butanol (w/w, 0.603 cm³, 40 μmol). The solution was stirred at room temperature for 45 min. Water (25 cm³) was added and the solution was extracted with dichloromethane (2 x 50 cm³). The organic phase was washed with a

saturated aqueous solution of sodium hydrogencarbonate ($3 \times 30 \text{ cm}^3$) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was redissolved in THF (12 cm^3) and water (12 cm^3). The mixture was stirred at room temperature and sodium borohydride (182 mg, 4.71 mmol) was added. After stirring 5 for 1.5 h, water (25 cm^3) was added and the solution was extracted with dichloromethane ($2 \times 50 \text{ cm}^3$). The organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate ($3 \times 30 \text{ cm}^3$) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give 10 **5** as a white solid material (1.13 g, 49%). $\delta_{\text{H}}(\text{CDCl}_3)$ 9.29 (1 H, br s, NH), 7.47 (1 H, d, J 1.1, 6-H), 7.38-7.25 (10 H, m, Bn), 6.22 (1 H, d, J 3.4, 1'-H), 4.62 (2 H, s, Bn), 4.60 (1 H, m, 4'-H), 4.46 (2 H, s, Bn), 4.35 (1 H, dd, J 3.4, 7.5, 2'-H), 3.83-3.73 (4 H, m, $2 \times$ 5'-H, $2 \times$ 2''-H), 2.67 (1 H, br s, OH), 2.07-2.01 (2 H, m, $2 \times$ 1''-H), 1.77 (3 H, d, J 0.5, CH_3). $\delta_{\text{C}}(\text{CDCl}_3)$ 164.3 (C-4), 150.3 (C-2), 137.6, 137.4 (Bn, C-6), 15 136.7 (Bn), 128.6, 128.4, 128.2, 127.8, 127.6, 127.3, 127.1 (Bn), 108.4 (C-5), 88.0, 87.7, 81.6, 74.7 (C-1', C-2', C-3', C-4'), 73.7, 69.6 (Bn), 64.6 (C-5'), 57.7 (C-2''), 28.6 (C-1''), 12.4 (CH_3). FAB-MS m/z 483 [M+H]⁺, 505 [M+Na]⁺ (Found: C, 63.6; H, 6.2; N, 5.4. $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}_7, 0.5\text{H}_2\text{O}$ requires C, 63.5; H 6.4; N, 5.7%).

20 **Example 9**

(*1S,5R,6R,8R*)-5-Hydroxy-6-(hydroxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]-octane (**6**). A solution of nucleoside **5** (1.08 g, 2.20 mmol) in anhydrous pyridine (5.0 cm^3) was stirred at 0 °C and a solution of *p*-toluenesulphonyl chloride (462 mg, 2.47 mmol) in anhydrous pyridine (2.0 cm^3) was added dropwise. After stirring at room 25 temperature for 20 h and addition of a mixture of water and ice (70 cm^3), extraction was performed with dichloromethane ($2 \times 75 \text{ cm}^3$). The organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate ($3 \times 50 \text{ cm}^3$) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give an intermediate which after evaporation was dissolved in anhydrous DMF (4.0 cm^3). The solution was added dropwise to a stirred suspension of 30 60% sodium hydride (203 mg, 4.94 mmol) in anhydrous DMF (4.0 cm^3) at 0 °C. The mixture was stirred for 18 h and water (20 cm^3) was added. After neutralisation with hydrochloric acid, dichloromethane (75 cm^3) was added. The organic phase was

washed with a saturated aqueous solution of sodium hydrogencarbonate ($3 \times 50 \text{ cm}^3$) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give a white solid material (858 mg). A solution of 5 this white solid material (846 mg, 1.80 mmol) in ethanol (10.0 cm^3) was stirred at room temperature and 20% palladium hydroxide over carbon (400 mg) was added. The mixture was degassed with argon and placed in a hydrogen atmosphere. After stirring for 2 h the mixture was directly purified by silica gel column chromatography using dichloromethane/methanol (97:3, v/v) as eluent to give 6 as a white solid 10 material (444 mg, 82%). δ_{H} ((CD_3)₂ SO) 11.3 (1 H, br s, NH), 7.36 (1 H, d, J 1.1, 6-H), 5.80 (1 H, d, J 4.3, 1'-H), 5.61 (1 H, s, OH), 4.86 (1 H, m, 5'-H_a), 3.89 (1 H, d, J 4.2, 2'-H), 3.85 (1 H, m, 2''-H_a), 3.83-3.64 (3 H, m, 4'-H, 5'-H_b, 2''-H_b), 2.14 (1 H, m, 1''-H_a), 1.81 (1 H, m, 1''-H_b), 1.78 (3 H, d, J 1.0, CH_3). δ_{C} (CD_3OD) 166.7 (C-4), 152.2 (C-2), 139.7 (C-6), 110.1 (C-5), 89.4, 89.1, 85.5, 85.2 (C-1', C-2', C-3', 15 C-4'), 71.4 (C-2''), 61.6 (C-5'), 37.0 (C-1''), 12.7 (CH_3) (Found: C, 47.4; H, 5.7; N, 9.0. $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_6\text{H}_2\text{O}$ requires C, 47.7; H, 6.0; N, 9.3%).

Example 10

(*1S,5R,6R,8R*)-6-(4,4'-Dimethoxytrityloxymethyl)-5-hydroxy-8-(thymin-1-yl)-2,7-dioxa-20 bicyclo[3.3.0]nonane (7). A solution of nucleoside 6 (310 mg, 1.09 mmol) in anhydrous pyridine (2.5 cm^3) was stirred at room temperature and 4,4'-dimethoxytrityl chloride (593 mg, 1.83 mmol) was added. After stirring for 3 h, additional 4,4'-dimethoxytrityl chloride (100 mg, 0.310 mmol) was added. After stirring for another 2 h, methanol (0.5 cm^3) was added and the mixture was evaporated. The residue was 25 dissolved in dichloromethane (5 cm^3) and washed with an aqueous saturated solution of sodium hydrogencarbonate ($3 \times 5 \text{ cm}^3$). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography with dichloromethane/methanol (99:1, v/v) as eluent to give 7 as a white solid material (618 mg, 97%). δ_{H} (CDCl_3) 9.04 (1 H, br s, NH), 7.47-7.16 (10 H, m, 6-H, DMT), 6.86-6.82 (4 H, m, DMT), 6.06 (1 H, d, J 4.1, 1'-H), 4.35 (1 H, d, J 4.1, 2'-H), 4.03 (1 H, m, 4'-H), 3.89 (1 H, m, 2''-H_a), 3.79 (6 H, s, 2 x OCH_3), 3.61 (1 H, m, 5'-H_a), 3.32-3.26 (2 H, m, 5'-H_b, 2''-H_b), 1.94-1.69 (2 H, m, 1''-H_a, 1''-H_b), 1.89 (3 H, s, CH_3). δ_{C} (CDCl_3) 163.4 (C-4), 158.6 (DMT), 150.1 (C-2), 144.3 (DMT), 137.2 (C-6), 135.6, 135.3, 129.9, 129.9, 128.9, 128.1, 127.9, 126.9, 125.2,

113.2 (DMT), 109.3 (C-5), 88.7, 87.3, 86.9, 83.5, 81.0 (DMT, C-1', C-2', C-3', C-4'), 69.7 (C-2''), 62.1 (C-5'), 55.1 (OCH₃), 36.5 (C-1''), 12.5 (CH₃).

Example 11

- 5 (**1S,5R,6R,8R**)-5-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-6-(4,4'-dimethoxy-trityloxymethyl)-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]nonane (**8**). A solution of nucleoside **7** (436 mg, 0.743 mmol) in anhydrous dichloromethane (2.2 cm³) and diisopropylethylamine (0.62 cm³) was stirred at room temperature and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.33 cm³, 1.46 mmol) was added. After 10 stirring for 1.5 h, methanol (0.4 cm³) and ethyl acetate (5 cm³) were added and the mixture was washed with aqueous saturated solutions of sodium hydrogencarbonate (3 x 5 cm³) and brine (3 x 5 cm³). The organic phase was dried (Na₂SO₄) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/triethylamine (97:3, v/v) as eluent, the 15 solvents were evaporated to give an oil which was dissolved in toluene (1 cm³) and precipitation from hexane at -30 °C yielded **8** as a solid white material (517 mg, 88%). δ_p (CDCl₃) 142.0, 141.9.

Example 12

- 20 **1-(3,5-Di-O-benzyl-3-C-(2-hydroxyethyl)-β-D-ribofuranosyl)thymine (9)**. To a stirred solution of nucleoside **2** (1.00 g, 2.09 mmol) in THF (5.4 cm³) and water (5.4 cm³) was added sodium periodate (1.34 g, 6.27 mmol) and a 2.5% solution of osmium tetroxide in *tert*-butanol (w/w, 0.265 cm³, 19 μmol). The solution was stirred at room temperature for 45 min. Water (25 cm³) was added and the solution was 25 extracted with dichloromethane (2 x 50 cm³). The organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 30 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was redissolved in THF (5.4 cm³) and water (5.4 cm³). The mixture was stirred at room temperature and sodium borohydride (79 mg, 2.08 mmol) was added. After stirring 30 for 1.5 h, water (25 cm³) was added and the solution was extracted with dichloromethane (2 x 50 cm³). The organic phase was washed with a saturated aqueous solution of sodium hydrogencarbonate (3 x 30 cm³) and dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give

nucleoside **9** as a white solid material (488 mg, 48%). δ_H (CDCl₃) 9.14 (1 H, br s, NH), 7.60 (1 H, d, J 1.1, 6-H), 7.40-7.22 (10 H, m, Bn), 6.25 (1 H, d, J 7.7, 1'-H), 4.59 (1 H, d, J 7.1 Bn), 4.49 (1 H, d, J 7.1 Bn), 4.39-3.30 (m, 8H, 4'-H, 2'-H, Bn, 5'-H_a, 5'-H_b, 2''-H_a, 2''-H_b), 2.23-2.00 (2 H, m, 1''-H_a, 1''-H_b), 1.49 (3 H, d, J 0.7, CH₃).
 5 δ_C (CDCl₃) 163.5 (C-4), 151.2 (C-2), 137.1, 136.5 (Bn), 135.7 (C-6), 128.7, 128.5, 128.2, 127.8, 127.6, 127.2 (Bn), 111.3 (C-5), 87.0, 82.7, 81.1, 78.3 (C-1', C-2', C-3', C-4'), 73.7, 69.6 (Bn), 64.4 (C-5'), 57.0 (C-2''), 32.4 (C-1''), 11.8 (CH₃) (Found: C, 63.9; H, 6.3; N, 5.4. C₂₆H₃₀N₂O₇, 0.25H₂O requires C, 64.1; H 6.3; N, 5.75%).

10 Example 13

1-[3-C-(2-O-t-Butyldimethylsilyloxyethyl)-3,5-di-O-benzyl-β-D-ribofuranosyl]thymine (10). A mixture of nucleoside **9** (1.80 g, 3.4 mmol) and t-butyldimethylsilyl chloride (0.585 g, 3.9 mmol) was dissolved in anhydrous pyridine (20 cm³). After 2 h at room temperature the reaction mixture was evaporated to dryness, twice co-evaporated
 15 with toluene (2 x 10 cm³) and re-dissolved in dichloromethane (150 cm³). The solution was washed with a saturated aqueous solution of sodium hydrocarbonate (2 x 50 cm³) and evaporated to give a foam. This material was purified by preparative silica-gel HPLC using gradient elution (0-3% methanol in dichloromethane, v/v) to give nucleoside **10** as a white solid material (1.86 g, 92%). δ_H (CDCl₃) 7.61 (1H, d, J 1.1, 6-H), 7.35-7.20 (10H, m, Bn), 6.27 (1H, d, J 7.9, 1'-H), 4.65-4.40 (4H, m, Bn, 2'-H), 4.37 (1H, s, Bn), 4.28 (1H, t, J 7.9, 4'-H), 4.35 - 3.55 (4H, m, 2''-H_a, 2''-H_b, 5'-H_a, 5'-H_b), 2.30-2.05 (2H, m, 1''-H_a, 1''-H_b), 1.46 (3H, s, 5-CH₃), 0.90 (9H, m, CH₃-C-Si), 0.08 (6H, m, CH₃-Si). δ_C (CDCl₃) 163.6 (C-6), 151.0 (C-2), 137.5, 136.6, 135.8 (C-5, Bn), 128.3, 128.1, 127.8, 127.2, 127.1, 126.8, 126.7 (Bn), 110.7 (C-4), 86.8,
 20 82.5, 81.6, 78.3 (C-1', C-2', C-3', C-4'), 73.3, 69.8 (Bn), 64.46 (C-5'), 58.2 (C-2''), 32.9 (C-1''), 25.6, 25.4, 17.9, -3.9, -5.7 (TBDMS), 11.6 (CH₃). FAB⁺-MS: m/z 597.19 [M+H]⁺, 619.18 [M+Na]⁺ (Found: C, 64.2; H, 7.4; N, 4.2; C₃₂H₄₄O₇N₂Si requires C, 64.4; H, 7.4; N, 4.7%).

30 Example 14

1-[3-C-(2-t-Butyldimethylsilyloxyethyl)-3,5-di-O-benzyl-β-D-erythro-pentofuran-2-ulosyl]thymine (11). A mixture nucleoside **10** (2.14 g, 3.59 mmol), 1.48 g (3.95 mmol) of pyridinium dichromate (1.48 g, 3.95) and activated 3A molecular sieve powder (4g) was suspended in anhydrous dichloromethane (80 cm³). After cooling the

mixture to -10 °C, acetic anhydride (10 cm³, 98 mmol) was added dropwise under vigorous stirring. The suspension was allowed to warm to room temperature and stirring was continued for 1.5 h whereupon the reaction was quenched by addition of triethylamine (20 cm³). The mixture was diluted with dichloromethane to 300 cm³ and 5 was washed with water (2 x 200 cm³). The organic phase was evaporated, and the residue purified by flash silica-gel chromatography using a gradient of 1.0, 1.2, 1.3, 1.4, 1.5% methanol in dichloromethane (v/v, total volume 250 cm³ each) to give nucleoside 11 (1.89 g, 84.4%) as a white solid material. δ_H (CDCl₃) 7.35-7.20 (1H, m, Bn, 6-H), 6.40 (1H, s, 1'-H), 4.57 (1H, s, Bn), 4.52 (1H, s, Bn), 4.46 (1H, d, J 11.0, Bn), 4.29 (1H, d, J 11.0, Bn), 4.07 (1H, dd, J' 0.5, 2.2, 4'-H), 3.95-3.70 (4H, m, 2''-H_a, 2''-H_b, 5'-H_a, 5'-H_b), 2.05 (1H, m, 1''-H_a), 2.42 (1H, m, 1''-H_b), 1.42 (3H, d, J 1.1, 5-CH₃), 0.86 (9H, s, CH₃-C-Si), 0.01 (6H, s, CH₃-Si). δ_C (CDCl₃) 202.6 (C-2'), 163.7 (C-4), 151.2 (C-2), 137.7, 136.6, 136.5 (Bn, C-6), 128.7, 128.5, 128.2, 128.1, 127.7, 126.4, 126.3 (Bn), 110.9 (C-5), 84.5, 81.3, 80.2 (C-1', C-3', C-4'), 15 73.6, 70.4 (Bn), 66.0 (C-5'), 57.6 (C-2''), 27.3 (C-1''), 25.9, 25.7, 18.2, -5.8, -5.9 (TBDMS), 11.7 (CH₃). FAB-MS *m/z* 595.14 [M+H]⁺ (Found: C, 64.1; H, 6.9; N, 4.5; C₃₂H₄₂O₂N₂Si requires C, 64.6; H, 7.1; N, 4.7%).

Example 15

20 (1*S*,5*R*,6*R*,8*R*)-1-Hydroxy-5-benzyloxy-6-benzyloxymethyl-8-(thymin-1-yl)-2,7-dioxa-bicyclo[3.3.0]octane (12). Compound 11 (1.80 g, 30.3 mmol) was dissolved in 0.5% HCl in methanol (w/w, 20 cm³) and the mixture was stirred for 30 min at room temperature. After evaporation to dryness, the residue was dissolved in dichloromethane (100 cm³) and washed with a saturated aqueous solution of sodium 25 hydrogencarbonate (2 x 40 cm³). The organic phase was evaporated and the residue was purified by flash silica-gel chromatography eluting with 2% methanol in dichloromethane (v/v) to yield nucleoside 12 (1.35 g, 93.5%) as a white solid material. δ_H (CDCl₃) 7.37-7.27 (1H, m, Bn, 6-H), 5.87 (1H, s, 1'-H), 4.71 (2H, s, Bn), 4.64 (1H, d, J 12.0, Bn), 4.56 (1H, d, J 12.0, Bn), 4.36 (1H, t, J 5.7, 4'-H), 4.16 (1H, m, 2''-H_a), 3.96 (1H, m, 2''-H_b), 3.74 (2H, m, 5'-H_a, 5'-H_b), 2.35-2.15 (2H, m, 1''-H_a, 1''-H_b), 1.88 (3H, s, CH₃). δ_C (CDCl₃) 163.7 (C-4), 151.4 (C-2), 137.8, 137.3, 136.7 (Bn, C-6), 128.5, 128.4, 128.0, 127.8, 127.5 (Bn), 109.9 (C-5), 108.6 (C-2'), 88.8, 87.1, 80.9 (C-1', C-3', C-4'), 73.6, 68.5, 68.1, 67.9 (C-5', C-2'', Bn), 30.9 (C-1''),

12.6 (CH₃). FAB-MS: *m/z* 481.03 [M+H]⁺, 503.02 [M+Na]⁺ (Found: C, 64.6; H, 5.8; N, 5.7; C₂₆H₂₈O₇N₂ requires C, 65.0; H, 5.9; N, 5.8%).

Example 16

5 (**1S,5R,6R,8R**)-1,5-Dihydroxy-6-hydroxymethyl-8-(thymin-1-yl)-2,7-dioxabicyclo-[3.3.0]octane (**13**). Compound **13** was successfully derived from compound **12** by catalytic removal of the benzyl protecting group in the same way as described in preparation of **6**. Purification of **13** was accomplished by column silica gel chromatography eluting with gradient concentrations (6 to 14%) of methanol in 10 dichloromethane. Analytical amounts of compound **13** (up to 15 mg) were additionally purified by reverse-phase HPLC at column (10 x 250 mm) packed by Nucleosil C18 (10 µm). Flow rate: 8 cm³/min; eluent: 0-10% acetonitrile in 60 min. Yield 82%. δ_H (CD₃OD) 7.44 (1H d, *J* 1.2, 6-H), 5.83 (1H, s, 1'-H), 4.10-3.80 (5H, m, 5'-H_a, 5'-H_b, 2''-H_a, 2''-H_b, 4'-H), 2.39-2.25 (1H, m, 1''-H_a), 2.00-1.90 (1H, m, 1''-H_b), 1.87 (3H, 15 d, *J* 1.2, CH₃). δ_C (CD₃OD) 166.3 (C-4), 152.7 (C-2), 139.8 (C-6), 110.0, 109.6 (C-2',C-5), 87.8, 85.8, 84.6 (C-1', C-3', C-4'), 68.8, 61.6 (C-5', C-2''), 35.6 (C-1''), 12.4 (CH₃). FAB-MS: *m/z* 301.03 [M+H]⁺ (Found: C, 46.6; H, 5.7; N, 8.5; C₁₂H₁₆O₇N₂ requires C, 48.0; H, 5.4; N, 9.3%).

20 Example 17

(**1S,5R,6R,8R**)-5-Benzylxyloxy-6-benzyloxymethyl-1-methoxy-8-(3-N-methylthymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (**14**), (**1S,5R,6R,8R**)-5-Benzylxyloxy-6-benzyloxymethyl-1-hydroxy-8-(3-N-methylthymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (**15**) and (**1S,5R,6R,8R**)-5-Benzylxyloxy-6-benzyloxymethyl-1-methoxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (**16**). A mixture of compound **12** (1.04 g, 2.16 mmol) and sodium hydride (171 mg of a 60% suspension in mineral oil, 4.30 mmol) was dissolved in anhydrous dichloromethane (4 cm³) during 10 min under stirring. Methyl iodide (1 cm³, 16 mmol) was added and the reaction mixture was incubated at 36 °C for 23 h. After evaporation, the residue was purified by silica gel column chromatography eluting with a gradient of 0.4-2.4% methanol in dichloromethane (v/v) to give products **14**, **15** and **16** and starting material **12** (212 mg, 20.5%). Compound **14** (47 mg, 4.3%). δ_H (CDCl₃) 7.25-7.37 (11H, m, Bn, 6-H), 6.15 (1H, s, 1'-H), 4.74 (1H, d, *J* 11.5, Bn), 4.67 (1H, d, *J* 11.3, Bn), 4.62 (1H, d, *J* 12.1, Bn), 4.55 (1H, d, *J* 11.9, Bn), 4.34 (1H, t, *J* 5.6, 4'-H), 3.99, (1H, m, 2''-H_a), 4.22 (1H, m, 2''-H_b), 3.72 (2H,

m, 5'-H_a, 5'-H_b), 3.41 (3H, s, CH₃-O), 3.35 (3H, s, CH₃-N³), 2.27 (1H, m, 1''-H_a), 2.41 (1H, m, 1''-H_b), 1.93 (3H, s, 5-CH₃). δ_c (CDCl₃) 163.3 (C-4), 151.0 (C-2), 138.2, 137.3, 135.7 (Bn, C-6), 128.3, 128.2, 127.8, 127.6, 127.4, 126.9 (Bn), 111.8 (C-5), 108.5 (C-2'), 89.1, 84.8, 79.5 (C-1', C-3', C-4'), 73.5, 68.4, 68.2, 67.3 (Bn, 5 C-5', C-2''), 50.8 (CH₃-O), 32.6 (C-1''), 27.9 (CH₃-N), 13.2 (CH₃). FAB-MS: *m/z* 508.88 [M+H]⁺ (Found: C, 65.7; H, 6.9; N, 4.8; C₂₈H₃₂O₇N₂ requires C, 66.1; H, 6.3; N, 5.5%). Compound 15 (97 mg, 9.1%). δ_H (CDCl₃) 7.37-7.28 (11H, m, Bn, 6-H), 5.86 (1H, s, 1'-H), 4.72 (2H, s, Bn), 4.64 (1H, d, *J* 11.9, Bn), 4.58 (1H, d, *J* 11.9, Bn), 4.37 (1H, t, *J* 5.6, 4'-H), 4.13 (1H, m, 2''-H_a), 3.93 (1H, m, 2''-H_b), 3.75 (2H, 10 m, 5'-H_a, 5'-H_b), 3.34 (1H, s, CH₃-N), 2.32-2.16 (2H, m, 1''-H_a, 1''-H_b), 1.93 (3H, s, CH₃). δ_c (CDCl₃) 163.2 (C-4), 151.9 (C-2), 137.5, 137.1, 134.0 (Bn, C-6), 128.4, 128.3, 128.1, 127.9 127.7, 127.6, 127.3 (Bn), 108.8, 108.5 (C-2', C-5), 88.7 (C-1'), 88.0, 81.0 (C-3', C-4'), 73.5, 68.3, 67.9, 67.7 (Bn, C-5', C-2''), 30.6 (C-1''), 27.8 (CH₃-N), 13.2 (CH₃). FAB-MS *m/z* 495.28 [M+H]⁺, 517.24 [M+Na]⁺.

15 Compound 16 (665 mg, 62.3%). δ_H (CDCl₃) 7.35-7.25 (11H, m, Bn, 6-H), 6.06 (1H, s, 1'-H), 4.73 (1H, d, *J* 11.5, Bn), 4.66 (1H, d, *J* 11.3, Bn), 4.61 (1H, d, *J* 11.9, Bn), 4.55 (1H, d, *J* 12.0, Bn), 4.34 (1H, t, *J* 5.6, 4'-H), 4.20 (1H, m, 2''-H_a), 3.98 (1H, m, 2''-H_b), 3.72 (2H, m, 5'-H_a, 5'-H_b), 3.40 (3H, s, CH₃-O), 2.45-2.35 (1H, m, 1''-H_a), 2.30-2.20 (1H, m, 1''-H_b), 1.90 (3H, d, *J* 1.1, CH₃). δ_c (CDCl₃) 163.2 (C-4), 150.1 (C-2), 138.2, 137.9, 137.3 (Bn, C-6), 128.4, 128.2, 127.8, 127.6 127.4, 127.1 (Bn), 110.8 (C-5), 109.3 (C-2'), 89.2, 84.2, 79.6 (C-1', C-3', C-4'), 73.6, 68.5, 68.3, 67.4 (Bn, C-5', C-2''), 50.8 (CH₃-O), 32.6 (C-1''), 12.5 (CH₃). FAB-MS *m/z* 495.22 [M+H]⁺, 517.23 [M+Na]⁺ (Found: C, 66.2; H, 7.2; N, 4.4; C₂₇H₃₀O₇N₂ requires C, 65.6; H, 6.1; N, 5.7%).

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Example 18

(1*S*,5*R*,6*R*,8*R*)-5-Hydroxy-6-hydroxymethyl-1-methoxy-8-(thymin-1-yl)-2,7-dioxabi-cyclo[3.3.0]octane (17). To a solution of nucleoside 16 (1.20 g, 2.43 mmol) in methanol (10 cm³) was added 20% palladium hydroxide over charcoal (250 mg) and 30 the mixture was carefully degassed under reduced pressure. An atmosphere of hydrogen was applied and stirring was continued for 12 h. The catalyst was removed by filtration of the reaction mixture through a glass column (1 x 8 cm) packed with silica gel in methanol. The column was additionally washed with methanol (20 cm³). All fractions were collected, evaporated to dryness and co-evaporated with petroleum

ether to yield a glass-like solid. This residue was purified by silica gel chromatography eluting with a gradient of 5-10% methanol in dichloromethane (v/v). The fractions containing the product were collected, combined and evaporated to dryness. The residue was dissolved in anhydrous methanol (5 cm³) and anhydrous benzene (100 5 cm³) was added. Lyophilisation yielded nucleoside 17 (0.61 g, 79%) as a white solid material. δ_H (CD₃OD) 7.45 (1H, s, 6-H), 5.93 (1H, s, 1'-H), 4.15-3.81 (5H, m, 5'-H_a, 5'-H_b, 2''-H_a, 2''-H_b, 4'-H), 3.43 (3H, s, CH₃-O), 2.47-2.40 (1H, m, 1''-H_a), 2.03-1.93 (1H, m, 1''-H_b), 1.92 (3H, s, CH₃). δ_C (CD₃OD) 164.1 (C-4), 150.1 (C-2), 138.3 (C-6), 109.6 (C-5), 108.3 (C-2'), 84.4, 84.1, 82.4 (C-1', C-3', C-4'), 68.0, 59.5 (C-5', C-10 2''), 49.6 (CH₃-O), 34.0 (C-1''), 10.5 (CH₃). FAB-MS *m/z* 315.13 [M+H]⁺, 337.09 [M+Na]⁺ (Found: C, 49.9; H, 5.7; N, 8.2; C₁₃H₁₈O₇N₂ requires C, 49.7; H, 5.8; N, 8.9%).

Example 19

15 (*1S,5R,6R,8R*)-6-(4,4'-Dimethoxytrityloxymethyl)-5-hydroxy-1-methoxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (18). A mixture of compound 17 (0.95 g, 3.03 mmol) and 4,4'-dimethoxytrityl chloride (1.54 g, 4.77 mmol) was dissolved in anhydrous pyridine (20 cm³) and stirred for 4 h at room temperature. The reaction mixture was evaporated to give an oily residue which was co-evaporated with toluene 20 (2 x 20 cm³). Dichloromethane (50 cm³) and a saturated aqueous solution of sodium hydrogencarbonate (50 cm³) were added, the organic phase was separated and evaporated, and the residue purified by silica gel HPLC (the residue was dissolved in the minimum amount of dichloromethane containing 0.5% triethylamine (v/v) and applied to the column equilibrated by the same solvent. The column was washed 25 (ethylacetate:petroleum ether:triethylamine; 15:84.5:0.5 (v/v/v, 1000 cm³) and the product was eluted in a gradient of methanol (0-2%) in dichloromethane containing 0.5% of triethylamine (v/v/v) to give compound 18 (1.71 g, 92.8%) as white solid material. δ_H (CDCl₃) 7.51-7.17 (10H, m, DMT, 6-H), 6.79-6.85 (4H, m, DMT), 6.04 (1H, s, 1'-H), 4.12-3.98 (3H, m, 5'-H_a, 5'-H_b, 4'-H), 3.77 (6H, s, CH₃-DMT), 3.49 (3H, s, CH₃-O), 3.45-3.32 (2H, m, 2''-H_a, 2''-H_b), 2.11-2.01 (1H, m, 1''-H_a), 1.94-1.87 (1H, m, 1''-H_b), 1.93 (3H, s, CH₃). δ_C (CDCl₃) 164.2 (C-4), 158.6, 144.7, 135.7, 130.1, 128.2, 127.9, 126.8, 113.2 (DMT), 150.7 (C-2), 137.7 (C-6), 109.8, 109.7 (C-5, C-2'), 86.5, 85.3, 85.0, 81.4 (DMT, C-1', C-3', C-4'), 69.2, 62.4 (C-5', C-2''), 55.2 (CH₃-DMT), 51.7 (CH₃-O), 35.5 (C-1''), 12.7 (CH₃). FAB-MS *m/z* 617.26

$[M + H]^+$, 639.23 $[M + Na]^+$ (Found: C, 66.4; H, 6.1; N, 4.2; $C_{34}H_{36}O_9N_2$ requires C, 66.2; H, 5.9; N, 4.5%).

Example 20

- 5 (**1S,5R,6R,8R**)-5-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-6-(4,4'-dimethoxytrityloxymethyl)-1-methoxy-8-(thymin-1-yl)-2,7-dioxabicyclo[3.3.0]octane (19). Compound **18** (1.2 g, 1.95 mmol) was dissolved in anhydrous dichloromethane (10 cm³). *N,N*-Diisopropylethylamine (1.35 cm³, 7.8 mmol) and 2-cyanoethyl-*N,N*-diisopropylphosphoramidochloridite (0.92 g, 3.9 mmol) were added under stirring at room temperature. After 72 h, the mixture was diluted to 100 cm³ by dichloromethane and washed by a saturated aqueous solution of sodium hydrogencarbonate (50 cm³). The organic phase was evaporated and applied to silica gel HPLC purification using a gradient of eluent B (petroleum ether:dichloromethane:ethyl acetate:pyridine; 45:45:10:0.5; v/v/v) in eluent A (petroleum ether:dichloromethane:pyridine; 50:50:0.5; v/v/v). The fractions containing the product were concentrated, co-evaporated with toluene (10 cm³) and dried under reduced pressure. The residue was dissolved in anhydrous benzene (20 cm³) and precipitated by addition of this solution into anhydrous petroleum ether (400 cm³) under stirring. The resulting white solid was isolated by filtration and dried to give compound **19** (0.96 g, 60.3%). δ_p ($CDCl_3$) 142.64, 142.52. FAB-MS *m/z* 817.26 $[M + H]^+$, 839.24 $[M + Na]^+$ (Found: C, 62.8; H, 6.4; N, 6.9; $C_{43}H_{53}O_{10}N_4P$ requires C, 63.2; H, 6.5; N, 6.9%).

Example 21

- 1,2-O-Isopropylidene-3-C-vinyl- α -D-ribofuranose (20).** A solution of 5-*O-t*-butyldimethylsilyl-1,2-*O*-isopropylidene- α -D-erythro-pent-3-ulofuranose (Y. Yoshimura, T. Sano, A. Matsuda, T. Ueda, *Chem. Pharm. Bull.*, 1988, **36**, 162) (6.05 g, 0.020 mol) in anhydrous THF (250 cm³) was stirred at 0 °C and a 1 M solution of vinylmagnesium bromide in ether (44 cm³, 44 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, whereupon saturated aqueous ammonium chloride (200 cm³) was added, and extraction was performed using dichloromethane (3 x 300 cm³). The combined extract was washed with brine (3 x 250 cm³) and dried (Na_2SO_4). The solvent was removed and the residue was redissolved in anhydrous THF (225 cm³). To this mixture was added a 1 M solution of tetrabutylammonium fluoride in THF (22 cm³, 22 mmol), stirring at room temperature was continued for 20 min